

Research Article

Intra- and interspecific pollen morphology variation of invasive *Reynoutria* taxa (Polygonaceae) in their response to different habitat conditions

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Abstract

Although understanding the relationship between the reproductive mode and mass-dispersal potential of plants is crucial for studying invasion phenomena, the morphological features of invasive species' pollen are not well understood. This study examined the pollen morphology and variability of three *Reynoutria* (knotweed) taxa (*R. japonica*, *R. sachalinensis*, *R. ×bohemica*) invasive in Europe, and their reaction to different habitat conditions within seven distinguished habitat types. The pollen was sourced from 95 sites from the taxa's invasive range in Central Europe. In total, 2850 pollen grains were measured and analysed for 11 quantitative and qualitative features. The pollen of *R. sachalinensis* was distinguished from that of the other two taxa (reticulate perforate) based on its rugulate and fossulate perforate exine ornamentation. The pollen's response to various habitat conditions, which was most marked in *R. ×bohemica*, was reflected by pollen size and exine thickness. Our research indicates that pollen availability is not a limiting factor for the sexual reproduction of knotweeds in Central Europe, including *Reynoutria japonica*, long considered male sterile. The observed presence of male-fertile specimens of *R. japonica* may enhance the efficiency of generative reproduction in this species throughout its invaded ranges. This finding should be considered when planning actions to control the population of these transformer plant species.

Key words: *Fallopia*, habitats, invasive plant species, pollen morphology, pollen variability, *Reynoutria*, SEM

Introduction

Taxa of the knotweed genus *Reynoutria* are currently considered to be among the most aggressive plant invaders in both Europe and North America, and have become one of the most intractable weeds to manage across the globe (Child and Wade 2000; Weber 2017; Cottet et al. 2020; Bzdęga et al. 2021; Hocking et al. 2023; Švec et al. 2024). Taxonomically, these robust Asian woody-stemmed herbaceous perennial plants belong to the subtribe Reynoutriineae in the Polygonaceae



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family of knotweeds from the order Polygonales, which includes, depending on the taxonomic approach, 40 (Balogh 2008) to 49 genera (Sanchez et al. 2011; Schuster et al. 2011). ‘Knotweed’ is a collective term used to refer to representatives of the genus *Reynoutria* Houtt. (syn. *Fallopia* Adans.) (= *Fallopia* sect. *Reynoutria* (Houtt.) Ronse De Craene). These strongly rhizomatous perennials include two species, *Reynoutria japonica* Houtt. (*Fallopia japonica* (Houtt.) Ronse Decr.) and *R. sachalinensis* (F. Schmidt) Nakai (*F. sachalinensis* (F. Schmidt) Ronse Decr.), and their hybrids, *R. ×bohemica* Chrtek & Chrtková (*Fallopia ×bohemica* (Chrtek & Chrtková) J.P. Bailey), as well as any backcrosses and hybrids resulting from crosses with other related species, including *F. baldschuanica* (Regel) Holub (sect. *Sarmientosae* (I. Grintz.) Holub) (Bailey and Wisskirchen 2006; Bailey et al. 2007, 2009; Hocking et al. 2019; Stace 2019; Hodálová et al. 2022).

Reynoutria japonica and *R. sachalinensis* are native to East Asia (Shaw 2008, 2013). The native range of *R. japonica* extends from the northernmost parts of the Sakhalin and the Kuril Islands, through Japan, Korea and Taiwan to Vietnam in the south (Bailey 2003; Balogh 2008; Alberternst and Böhmer 2011). The area of natural occurrence of *R. sachalinensis* is much smaller, covering Sakhalin, the southern Kurils, Japan (northern Hokkaido and part of Honshu) and Ullung Island (Bailey and Conolly 2000; Balogh 2008). In recent decades, *R. japonica* and *R. sachalinensis* have spread outside of their native distribution range into Europe, North America, Australia and New Zealand (Gibbs et al. 1987; Beerling et al. 1994; Sukopp and Starfinger 1995; Owen 1996; Bailey and Wisskirchen 2006; Barney et al. 2006; Bailey et al. 2007, 2009; Shaw 2008, 2013; Tokarska-Guzik et al. 2017). In addition, *R. japonica* occurs in South America (Chile) (Saldaña et al. 2009) and *R. sachalinensis* was found in South Africa (Shaw 2013). In Europe, these species were introduced in the 19th century as decorative garden plants and spread rapidly into natural habitats (Bailey and Conolly 2000; Bailey 2013). In 1983, in what was then Czechoslovakia, a hybrid of two species, *Reynoutria ×bohemica*, was first observed and described (Chrtek and Chrtková 1983). After this publication, sites of the hybrid were found in other European countries (Bailey et al. 1995; Keil and Alberternst 1995; Fojcik and Tokarska-Guzik 2000; Balogh 2008), and its presence was also confirmed in the area of natural occurrence of knotweed in Japan (Bailey 2003).

In their native ranges, *R. japonica* and *R. sachalinensis* propagate both sexually and vegetatively (Shaw 2008, 2013). In invaded ranges, it has been assumed that all knotweed taxa mainly reproduce vegetatively and disperse clonally by the growth/regeneration of rhizomes and shoots, and by the fragmentation of these organs (e.g. Brock et al. 1995; Shaw and Seiger 2002; Bailey et al. 2009; Alberternst and Böhmer 2011; Strgulc Krajšek and Dolenc Koce 2015). Despite the development of viable seeds (Forman and Kesseli 2003; Strgulc Krajšek and Dolenc Koce 2015) and the normal growth and development of knotweed seedlings in laboratory conditions (Bailey 1994; Forman and Kesseli 2003; Engler et al. 2011; Kadlecová et al. 2024), in the wild successful sexual reproduction of these taxa was considered rather rare (Pyšek et al. 2003; Tiébré et al. 2007; Bailey et al. 2009; Engler et al. 2011). However, according to some authors, in the case of *R. ×bohemica*, sexual reproduction is the main factor determining its invasive character (Buhk and Thielsch 2015; Strgulc Krajšek and Dolenc Koce 2015; Tokarska-Guzik et al. 2017). Hybrids are most often produced from seeds developing on *R. japonica*

shoots. This is the result of pollination of the ‘pistillate’ flowers with pollen from *R. sachalinensis* flowers or related species (Bailey 2013; Strgulc Krajšek and Dolenc Koce 2015; Tokarska-Guzik et al. 2017).

Knotweeds are described as dioecious plants, characterized by the presence of two groups of individuals in one species: one produces hermaphroditic flowers, and the other produces female flowers (male sterile, so-called gynodioecia) (Bailey 1994; Bailey et al. 1996; Forman and Kesseli 2003; Alberternst and Böhmer 2011). The breeding system of *R. japonica* in Europe has been described as dioecious and gynodioecious (Bailey 1994), but all plants were found to be functionally female (male-sterile) (Bailey 1994; Beerling et al. 1994; Hollingsworth and Bailey 2000). This caused European populations of this plant to be considered as a genetically uniform single female clone (Bailey et al. 2009). However, there are some indications that due to the different histories and dynamics of *R. japonica* invasions in Western and Central Europe and North America, invasive populations of these plants may have other characteristics (Bzdęga et al. 2012). Although genetic analyses confirmed the lack of genetic diversity of the *R. japonica* population in Britain (Hollingsworth and Bailey 2000), such variation was found in populations in North America (Grimsby et al. 2007) and between individuals of *R. japonica* in Central Europe (Bzdęga et al. 2012; Meredá et al. 2023). This diversity may be the result of episodes of sexual reproduction (Bzdęga et al. 2012), i.e. the occurrence of male flowers with pollen.

In their native range, *Reynoutria japonica* and *R. sachalinensis* show high tolerance to environmental factors and have similar soil requirements and preferences for soil reaction (Shaw 2008, 2013). Both species most often occur in open and sunny habitats and, as pioneer species, often become established on the slopes of volcanoes. *Reynoutria sachalinensis* grows on mountain landslides and areas along ravines and mountain streams, encroaches on seaside cliffs, and is also found on the edges of forests, whereas *R. japonica* prefers open and humid areas, where, like *R. sachalinensis*, it usually grows on sunny slopes of hills and forest edges; more often, however, it is found on the banks of ditches and roadsides (Bailey and Conolly 2000; Bailey 2003).

To date, the pollen morphology of species belonging to several genera from the family Polygonaceae has been described. The most frequently described genera among them are *Rumex* and *Polygonum*, then, much less frequently, *Atraphaxis*, *Persicaria*, *Polygonella*, *Avicularia*, *Pseudomollia* or *Duravia* (e.g. Nowicke and Skvarla 1977; Zhong-ze et al. 1999; Hong et al. 2005; Yasmin et al. 2010a, Yasmin et al. 2010b; Mosaferi and Keshavarzi 2011; Yurtseva et al. 2014; Paul and Chowdhury 2020; Kong et al. 2021; Raycheva et al. 2021; Başer et al. 2022), while studies on the genera *Fallopia* and *Reynoutria* are relatively sparse (Halbritter 1998; Yasmin et al. 2009; Heigl 2020, 2021; Paul and Chowdhury 2020). Moreover, no studies on the variability of pollen grains of these plants have been conducted to date.

The taxonomic utility of pollen characters of *F. convolvulus* and *F. dumetorum* from Pakistan was shown in the study of Yasmin et al. (2009). Brief descriptions of pollen grains of four *Reynoutria* and *Fallopia* species (*F. aubertii*, *F. baldschuanica*, *F. convolvulus*, *F. japonica* (*R. japonica*)) were provided by Halbritter (1998) and Heigl (2020, 2021). Paul and Chowdhury (2020) characterised pollen grains of *F. convolvulus* as part of a broader study on the pollen morphology of 32 taxa of subfamily Polygonoideae (Polygonaceae) from India.

Reynoutria japonica, *R. sachalinensis* and *R. ×bohemica* are considered transformer plant species in Europe (Chmura et al. 2015; Protopopova et al. 2015; Gentili et al. 2022). However, sexual reproduction is rarely observed in these taxa, which may be due to their pollen characteristics. Moreover, they grow in many different habitats (Bímová et al. 2004; Tiébré et al. 2008) that may play an important role in mediating the sexual recruitment of the plants. Consequently, the results of our research have an important practical aspect, as the reaction of the pollen of these invasive taxa to changing habitat conditions may affect the quality and effectiveness of their sexual reproduction.

Based on these premises, the primary objective of this study was to present a complete description of the pollen morphology of three *Reynoutria* taxa (*R. japonica* and *R. sachalinensis*, and their hybrids *R. ×bohemica*) – as was not found in the palynological literature – and investigate the range of intraspecific and interspecific variability of pollen grains of these invasive taxa, which has not been analysed to date. We also considered the influence of habitat conditions on the pollen morphology and variability and the share of male-fertile pollen-setting individuals in the Central European populations of the studied taxa. We tested the hypothesis that (1) there are individuals that set pollen in the studied populations of all *Reynoutria* taxa in Central Europe, and that (2) habitat conditions influence the morphological structure of knotweed pollen.

Material and methods

Plant material identification, collection and palynological analysis

The plant material (inflorescences) of three studied taxa: *Reynoutria japonica*, *R. sachalinensis* and *R. ×bohemica* was collected from August to October 2021, on dates of the optimal development phase of plants of individual taxa. The inflorescences were collected from 95 localities in the invasion range in Poland (*R. japonica* – 41 samples, *R. sachalinensis* – 30 samples and *R. ×bohemica* – 24 samples (Appendices 1, 2)), which corresponds to the range of occurrence of individual *Reynoutria* taxa in the study area (Zajac and Zajac 2001, 2019). The selection of localities was preceded by field reconnaissance conducted in previous years (some sites were recognized during earlier research), and data on the distribution of knotweeds collected from available sources, such as published research (e.g. Tokarska-Guzik et al. 2010; Bzdęga et al. 2012, 2022), herbarium data (e.g. Tokarska-Guzik et al. 2017) and unpublished data (mainly original author's data). Regarding population size, similar stands of *Reynoutria* taxa were selected for the study, although minor regional variations in the area occupied by each taxon favor *R. japonica* and *R. ×bohemica*. The determination of the knotweed was based on their morphological characteristics such as leaf shape and size, trichome type and morphology, flower number in the bunch, length of the stamens in relation to the perianth and the size of the panicle, which are well described as traits that are diagnostic for the identification of *Reynoutria* taxa (Beerling et al. 1994; Fojcik and Tokarska-Guzik 2000; Bailey et al. 2009; Tokarska-Guzik et al. 2017; Meredá et al. 2019; Bzdęga et al. 2022). At each site, five ramets from several clumps were selected, and from each of them, three inflorescence clusters were collected. The collected plant material was stored at the Department of Botany and Forest Habitats at the Poznań University of Life Sciences.

In accordance with the study by Wrońska-Pilarek et al. (2015), each sample ($n = 95$) consisted of 30 randomly selected, mature and correctly formed pollen grains. The pollen grains were prepared for light microscopy (LM) and scanning electron microscopy (SEM) using the acetolysis method described by Erdtman (1952, 1960). The inflorescences were placed in tubes and then centrifuged with glacial acetic acid. Pollen grains were mixed with the acetolysis solution, which consisted of nine parts acetic anhydride and one part concentrated 95% sulphuric acid. The mixture was then heated to boiling and kept in a water bath for 2–3 min. Samples were centrifuged in the acetolysis mixture, washed with acetic acid and centrifuged again. The pollen grain samples were then mixed with 96% ethyl alcohol and centrifuged four times, with processed grains subsequently divided into two parts: one half of the samples was immersed in glycerin jelly for LM, while the other was placed in 96% ethyl alcohol in preparation for SEM. The pollen morphological observations were carried out using a scanning electron microscope (Zeiss Evo 40) and a digital light microscope (Levenhuk D870T) equipped with a camera and software enabling accurate measurements of the pollen grain.

We analysed the pollen grains for 11 quantitative characteristics: length of the polar axis (P) and equatorial diameter (E), length of the ectoaperture (Le), the distance between apices of two ectocolpi (d), thickness of the exine along the polar axis (Exp), thickness of the exine along the equatorial axis (Exe), as well as the ratios P/E, Le/P, Exp/P, Exp/E and d/E (apocolpium index P.A.I). The pollen shape classes (LA/SA ratio) were adopted based on the classification proposed by Erdtman (1952): suboblate (0.75–0.88), oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), subprolate (1.15–1.33), prolate (1.34–2.00) and perprolate (> 2.01). The following qualitative features were also analysed: pollen outline and shape, exine ornamentation, operculum structure.

To estimate the amount of pollen in the tested plants, the pollen grain samples prepared for digital LM were used. Using a single-channel pipette, 0.5 ml of the glycerin jelly containing pollen grains from all pollen samples ($n = 95$) was transferred to a glass microscope slide, and a coverslip was applied. A digital LM (Levenhuk D870T) was used to count the number of pollen grains in all samples. As most samples contained more than 1000 pollen grains, the amount of pollen was subsampled (in all samples, all pollen grains were counted for ten areas of 1 mm^2 , evenly distributed over the coverslip).

The palynological terminology used in the study follows Punt et al. (2007) and Halbritter et al. (2018).

Habitat analysis

The habitat type was defined at each site from which plant material was collected based on expert knowledge. Due to the great diversity of these areas, the habitats were grouped into seven categories corresponding to the plant communities of Matuszkiewicz (2022) (Table 1). The majority of samples were gathered from typical anthropogenic sites (60), mainly roadsides and railway embankments (42 samples), and other areas, including various types of urban and post-industrial wastelands, parks and gardens (18). The second group consisted of samples collected from semi-natural habitats, such as non-forested edges of large and small rivers and drainage ditches – hereinafter referred to as the edges of watercourses (22) – abandoned meadows (7) and forests (6) (in order of increasing habitat fertility: mesic mixed coniferous forest (1), mesic mixed broadleaved forest (2), mesic broadleaved forest (1) and floodplain forest (2)).

Table 1. Types of habitats from which the *Reynoutria* plant material originated, with corresponding classes of plant communities according to Matuszkiewicz (2022).

No.	Habitat type	Classes of plant communities corresponding to habitats	<i>R. japonica</i>	<i>R. sachalinensis</i>	<i>R. ×bohemica</i>
1	anthropogenic roadsides and other anthropogenic sites	<i>Stellarietea mediae</i> R.Tx., Lohm. et Prsg. 1950, <i>Artemisietea vulgaris</i> Lohm., Prsg. et R.Tx. in R.Tx. 1950	29	22	9
2	abandoned meadows and wastelands	<i>Molinio-Arrhenatheretea</i> R.Tx. 1937, <i>Artemisietea vulgaris</i> Lohm., Prsg. et R.Tx. in R.Tx. 1950, <i>Stellarietea mediae</i> R.Tx., Lohm. et Prsg. 1950	1	4	2
3	edge of the watercourses	<i>Phragmitetea australis</i> R.Tx. et Prsg. 1942, <i>Scheuchzerio-Caricetea nigrae</i> (Nordh. 1937) R.Tx. 1937, <i>Artemisietea vulgaris</i> Lohm., Prsg. et R.Tx. in R.Tx. 1950, <i>Stellarietea mediae</i> R.Tx., Lohm. et Prsg. 1950	9	2	11
4	mesic mixed coniferous forest	<i>Vaccinio-Piceetea</i> Br.-Bl. 1939, <i>Quercetea robori-petraeae</i> Br.-Bl. et R.Tx. 1943	1	0	0
5	mesic mixed broadleaved forest	<i>Quercu-Fagetea</i> Br.-Bl. et Vlieg. 1937 (moderately fertile communities)	0	1	1
6	mesic broadleaved forest	<i>Quercu-Fagetea</i> Br.-Bl. et Vlieg. 1937 (fertile communities)	0	1	0
7	floodplain forest	<i>Salicetea purpureae</i> Moor 1958, <i>Quercu-Fagetea</i> Br.-Bl. et Vlieg. 1937	1	0	1
Total number of samples			41	30	24

Statistical analysis

One-way ANOVA with Tukey’s post hoc test was used to compare the number of pollen grains in the studied samples (logarithm-transformed) between three *Reynoutria* taxa. Multivariate analysis of variance (MANOVA) was conducted to test for differences in three *Reynoutria* taxa as well as between habitat types for each *Reynoutria* taxon separately. In the MANOVA, an error structure including independent variables within the samples was added. Next, linear discriminant analysis (LDA) was used to analyse variation within and between *Reynoutria* taxa and habitat types. Box’s M test was used to verify the multivariate normality and homogeneity of variance–covariance matrices. Next, mixed ANOVA’s with Tukey’s post hoc test were used to compare pollen traits among the three *Reynoutria* taxa and habitats. The Bonferroni correction method was used for multiple comparisons of habitats. The samples were included as a random effect on each model to control for the non-independence of the site where pollen grains were collected. Shapiro–Wilk’s normality test was used to verify whether the residuals were normal for each pollen trait. The homogeneity of variance was tested using Bartlett’s test; all the pollen traits were found to have a normal distribution. Although mixed ANOVA is generally robust to moderate imbalances, extreme disparities in sample sizes can lead to inflated Type I error rates or reduced statistical power, especially in pairwise comparisons. To mitigate this, we applied a Bonferroni correction for multiple comparisons, which helps control for the risk of false positives arising from the imbalanced samples. Moreover, the relationships between the 11 observed traits were estimated using Pearson’s linear correlation coefficients based on the means for *R. japonica*, *R. sachalinensis* and *R. ×bohemica* separately. The significant relationship between traits ($p < 0.05$) was presented on heat maps. The analyses were carried out in R 4.0.2 (R Core Development Team 2020): the linear discriminant analysis was carried out using the MASS package (Venables and Ripley 2002), the mixed ANOVA using the lme4 (Bates et al. 2015) and car (Fox and Weisberg 2011) packages, and the data visualisation using the ggplot2 package (Wickham 2016).

Results

Number of pollen grains in the studied samples

All the tested specimens of knotweed ($n = 95$) developed male-fertile flowers containing pollen. Pollen grains were found in 41 specimens of *R. japonica*, 30 specimens of *R. sachalinensis* and 24 specimens of *R. ×bohemica*. The total number of pollen grains on a 10-mm² surface was 1–622 (5.79 ± 16.53) in *R. japonica*, 3–807 (217.54 ± 276.77) in *R. ×bohemica* and 1–1560 (236.57 ± 331) in *R. sachalinensis*.

The ANOVA indicated significant differences in the number of pollen between the three *Reynoutria* taxa ($F_{2,92} = 15.78$, $p < 0.0001$). Tukey post hoc tests indicated significant differences between *R. japonica* and both other taxa (Fig. 1). The least-numerous pollen samples were found in *R. japonica*, where 68.3% of samples had 5 or fewer pollen grains across a 10-mm² surface, versus 30% in *R. sachalinensis* and 12.5% in *R. ×bohemica*. At the same time, the lowest number (9.8% – 4 specimens) of samples containing over 100 pollen grains was found in *R. japonica*, versus 41.7% in *R. ×bohemica* and 50% in *R. sachalinensis* (Fig. 1).

General morphological description of pollen

In total, 2850 *Reynoutria* pollen grains were analysed (1230 of *R. japonica*, 900 of *R. sachalinensis* and 720 of *R. ×bohemica*). The studied pollen grains were tricolporate, isopolar monads (Fig. 2A–J, Table 2), mostly small (10–25 µm; 70%) and rarely medium sized (25.1–50 µm; 30%), with small pollen grains accounting for 81% of pollen in *R. sachalinensis*, 67% in *R. japonica*, and 58% in *R. ×bohemica*.

The mean length of the polar axis (P) was 23.88 (11.90–44.15) µm. As the analysed pollen had a very small range of average values for trait P (from 23.26 to 24.52 µm), most of the *Reynoutria* pollen grains belonged to the upper limit of small pollen. The hybrid (*R. ×bohemica*) developed longer and wider pollen grains than the parental species *R. japonica* and *R. sachalinensis*. The values of trait P were 23.26 (14.96–44.15) µm in *R. sachalinensis*, 23.97 (16.09–40.33) µm in *R. japonica* and 24.52 (11.90–37.50) µm in *R. ×bohemica* (Table 2).

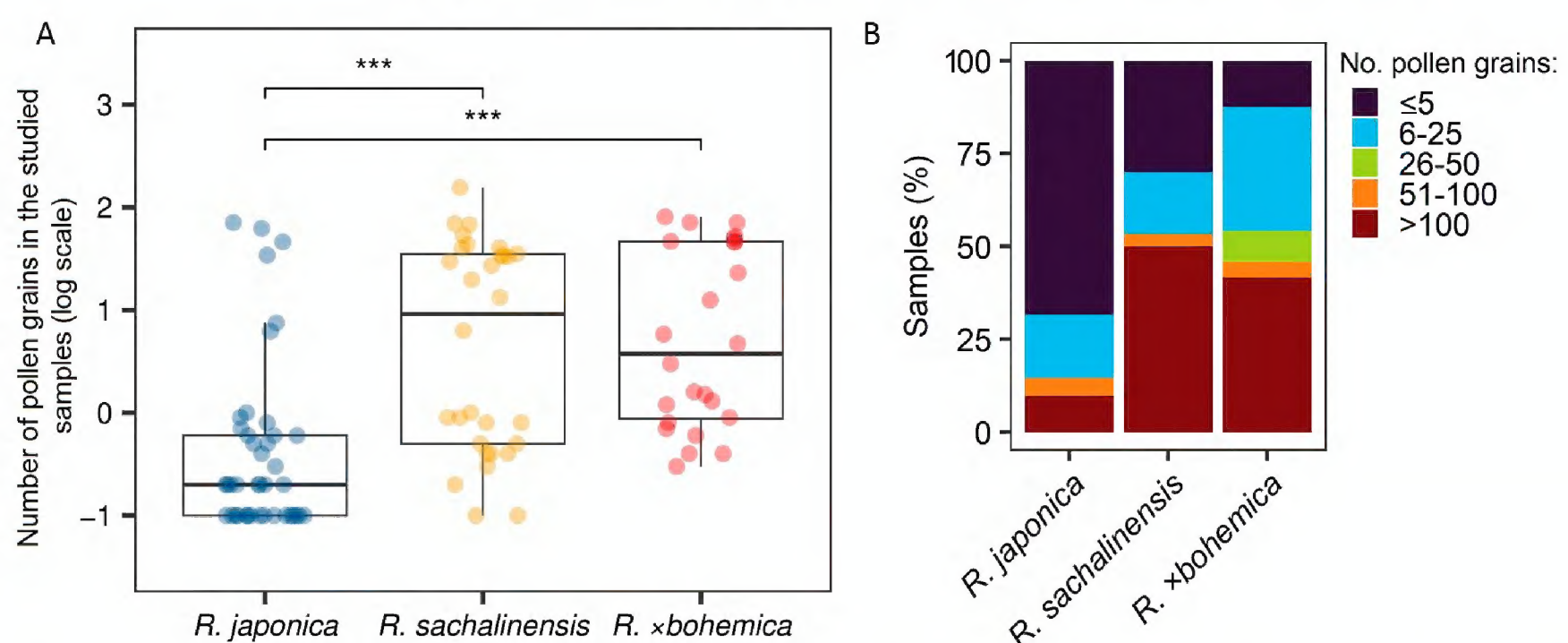


Figure 1. A boxplot of the number of pollen grains in the studied samples (log scale) of *Reynoutria* taxa B frequency of number of pollen-grain categories in the studied samples of *Reynoutria* taxa. Each point represents a single observation. Asterisks indicate significant differences (***P < 0.001).

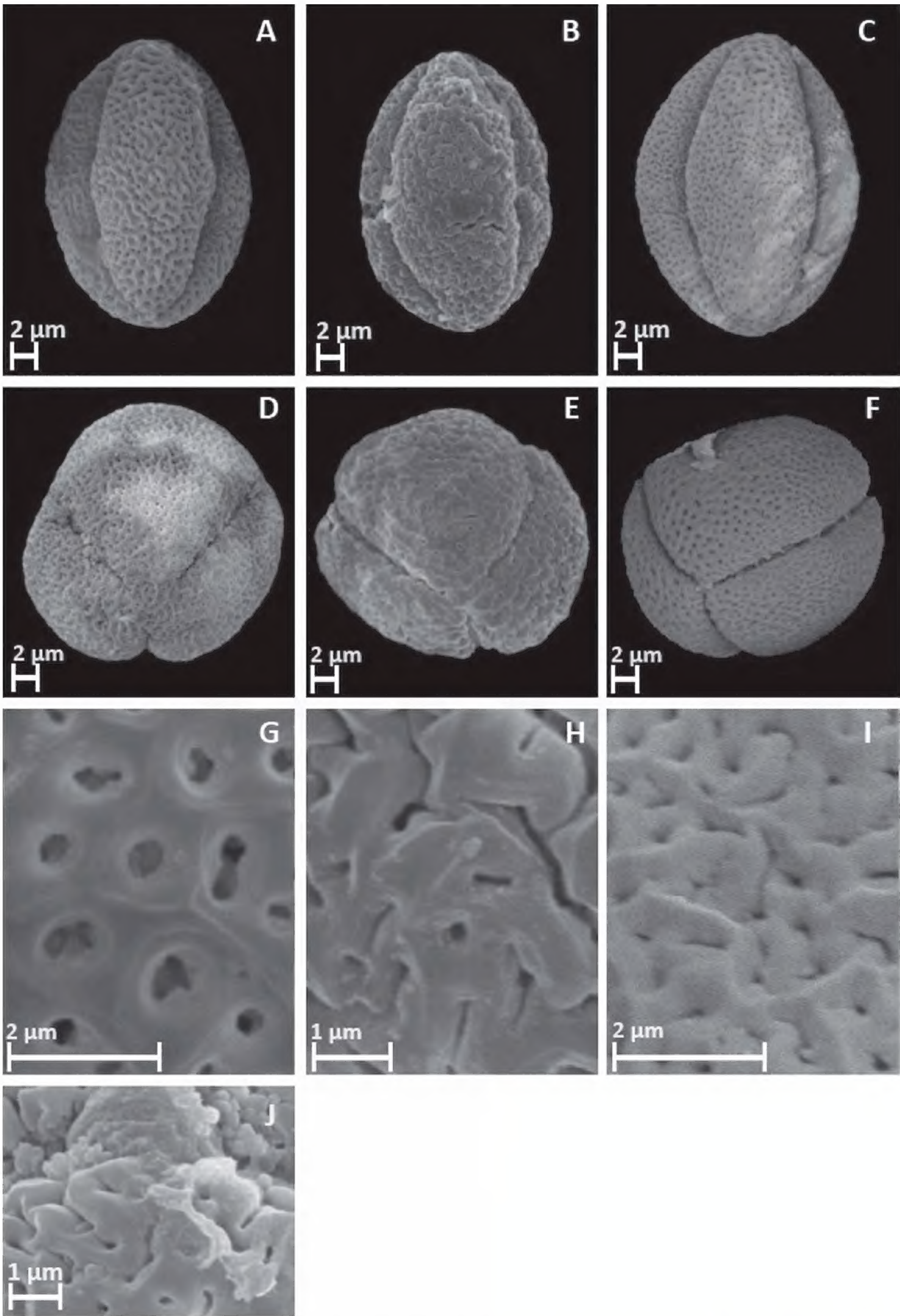


Figure 2. Pollen equatorial and polar views and exine ornamentation under scanning electron microscopy (SEM) **A–C** equatorial view of *R. japonica*, *R. sachalinensis*, *R. xbohemica* **D–F** polar view and three colpi of *R. japonica*, *R. sachalinensis*, *R. xbohemica* **G–I** exine ornamentation of *R. japonica*, *R. sachalinensis*, *R. xbohemica* **J** operculum in *R. sachalinensis*.

The mean value of trait E was 22.91 (9.73–36.47) μm . The values of this trait were 23.62 (11.06–34.05) μm in *R. xbohemica*, 22.87 (9.73–36.47) in *R. japonica* and 22.40 (10.97–34.32) μm in *R. sachalinensis*. The narrowest range of E variation was found in *R. japonica* (16.09–40.33 μm), and the widest in *R. sachalinensis* (14.96–44.15 μm) (Table 2). The outline in the polar view was mostly circular, and in the equatorial view mostly elliptic and rarely circular (Fig. 2A–F).

Table 2. The results of the mixed ANOVAs for pollen grain trait differences between studied *Reynoutria* taxa.

	<i>R. japonica</i>					<i>R. sachalinensis</i>					<i>R. ×bohemica</i>					F	df	p
	mean	SD	min	max	CV (%)	mean	SD	min	max	CV (%)	mean	SD	min	max	CV (%)			
P	23.969	3.125	16.090	40.330	13.04	23.264	2.603	14.960	44.150	11.19	24.518	3.005	11.900	37.500	12.26	7.14	2,2850	0.001
Le	19.447	3.033	7.350	32.720	15.60	19.224	2.330	10.180	39.150	12.12	19.582	2.774	7.900	31.650	14.17	0.68	2,2850	0.51
d	7.914	1.782	3.070	17.430	22.51	7.359	1.646	3.340	16.260	22.37	8.055	2.399	2.230	17.730	29.78	5.92	2,2850	0.004
E	22.866	3.068	9.730	36.470	13.42	22.399	2.386	10.970	34.320	10.65	23.624	3.015	11.060	34.050	12.76	7.08	2,2850	0.001
Exp	1.369	0.244	0.850	2.120	17.80	1.445	0.291	0.670	2.350	20.17	1.408	0.314	0.630	2.600	22.31	3.17	2,2850	0.046
Exe	1.482	0.235	0.940	2.180	15.86	1.607	0.294	0.820	2.500	18.30	1.460	0.356	0.480	2.400	24.37	8.34	2,2850	<0.001
P/E	1.053	0.095	0.691	2.382	9.05	1.042	0.094	0.764	1.881	9.00	1.044	0.112	0.711	1.745	10.72	1.57	2,2850	0.21
Le/P	0.811	0.060	0.341	1.000	7.42	0.827	0.052	0.429	1.000	6.29	0.799	0.061	0.381	1.000	7.58	11.5	2,2850	<0.001
d/E	0.347	0.069	0.163	0.887	20.01	0.329	0.066	0.153	0.601	20.19	0.340	0.090	0.128	0.699	26.42	2.93	2,2850	0.05
Exp/P	0.058	0.012	0.029	0.112	20.59	0.062	0.012	0.029	0.101	19.51	0.058	0.014	0.025	0.119	24.22	7.01	2,2850	0.002
Exp/E	0.061	0.013	0.033	0.161	21.64	0.065	0.013	0.030	0.112	20.04	0.060	0.015	0.026	0.128	25.29	5.38	2,2850	0.006

The P/E ratio (pollen shape) varied, averaging 1.05 (0.69–2.38). *Reynoutria* pollen grains were mostly prolate spherical (55.1% – 1570 pollen grains) or oblate spherical (30.8% – 879), rarely subprolate (12% – 342), and very rarely suboblate (1% – 28), prolate (0.9% – 27), oblate (0.1% – 3) or perprolate (0.04% – 1). Very similar results were obtained when analysing the distribution of pollen shape classes in individual taxa. In each of the taxa, prolate spherical pollen was found to be the most numerous (*R. japonica* – 59.9%, *R. sachalinensis* – 53.1%, *R. ×bohemica* – 43.1%), while oblate spheroidal pollen was the second most abundant shape, followed by subprolate pollen (Table 2).

The mean apocolpium index P.A.I (d/E ratio) was 0.34 and ranged from 0.13 (in *R. ×bohemica*) to 0.89 (in *R. japonica*). The lowest mean values of this index were recorded in *R. sachalinensis* (0.33), while the highest, which occurred in *R. japonica*, were similar (0.35) (Table 2).

The mean exine thickness was 1.46 (0.48–2.6) µm. The exine was thickest in *R. sachalinensis* (Exp – 1.45 µm and Exe – 1.61 µm) and thinnest in *R. japonica* (Exp – 1.37 µm) and *R. ×bohemica* (Exe – 1.46 µm). The mean exine thickness consisted of about 0.058 (0.02–0.12) of the P and 0.08 (0.02–0.14) of the E. The similarity of these results indicated a more or less equal exine thickness along the ends, thick regular with an irregular course and thick, slightly flattened walls of lumina, and lumina with small diameters in the pollen grains (Table 2).

Pollen grains usually possess three apertures, or ‘colpori’. Colpi were arranged meridionally, regularly, were more or less evenly spaced, and were usually long (19.41 (7.35–39.15) µm) and very narrow, usually widening in the central (pori) area (Fig. 2A–F, Table 2). On average, the length of colpi constituted 81% of the polar axis length. Sometimes the colpi were so long that they met in the polar area, as in *R. ×bohemica* (Fig. 2A–F). The shortest colpi were found in *R. ×bohemica* (7.35 µm), while the longest was found in *R. sachalinensis* (39.15 µm). Sculpturing of ectocolpus membranes was close to microgranulate (Fig. 2J). The operculum observed in the studied taxa was minor, elliptical or spheroidal, slightly convex and situated above the porus, usually in the central part of the ectocolpus. Its sculpture was psilate and often corrugated (Fig. 2J).

Under SEM, the exine ornamentation of *R. sachalinensis* was rugulate, fossulate perforate, while that of *R. japonica* and *R. ×bohemica* was reticulate perforate (Fig. 2G–I). In *R. sachalinensis*, rugulae or fossulae were elongated, wide, flattened

with irregular, geometric outlines and irregularly arranged. The perforations were small, quite sparse and irregularly distributed with different diameters (usually 0.1–0.2 μm). The reticulum of *R. \times bohemica* had wide muri – which were flattened at the top and had an irregular course – and lumina of various diameters and irregular outlines. The perforations were located at the bottom of the lumina and were very numerous, small and of similar diameter (usually 0.2 μm), as well as usually appearing circular or elliptical in outline. The reticulum of *R. japonica* consisted of wide, irregularly-shaped muri with an irregular, undulating course and walls narrowing at the top. The lumina had irregular outlines and varied diameters (usually 1.5 μm), usually larger than those in *R. \times bohemica*. Very numerous perforations were located at the bottom of the lumina, with quite large diameters (usually 0.20–0.40 μm , up to 0.80 μm), larger than those in *R. \times bohemica*.

The MANOVA revealed clear-cut, significant differences in terms of pollen grain traits between studied *Reynoutria* taxa ($F = 7.67$, Pillai = 1.01, $p < 0.001$). The mixed ANOVA also indicated significant differences in pollen traits between *Reynoutria* taxa, except for Le and P/E (Fig. 3, Table 2). Tukey's post hoc tests indicated significant differences in P, d, E, Exe, Le/P, Exp/P and Exp/E between *R. \times bohemica* and *R. sachalinensis*, and in P, d, Exe, Exp, d/E, Le/P, Exp/P and Exp/E between *R. japonica* and *R. sachalinensis* (excluding E). E significantly differed between *R. \times bohemica* and *R. japonica*.

The LDA showed that *R. sachalinensis* was more separate from *R. \times bohemica* and *R. japonica* (Fig. 4). The first two linear discriminant (LD) functions accounted for 100% of the total variability between the three *Reynoutria* taxa.

The most significant positive, linear relationships with the first LD were found for Exe (0.508) and Le/P (0.574), while a negative relationship was found for P (0.469) (Figs 5–7). The second LD was significantly positively correlated with E (0.242) and Exp (0.272), and negatively correlated with P/E (0.241). For *R. japonica*, strong significant positive correlations were found between Le and P (0.90), E and P (0.95),

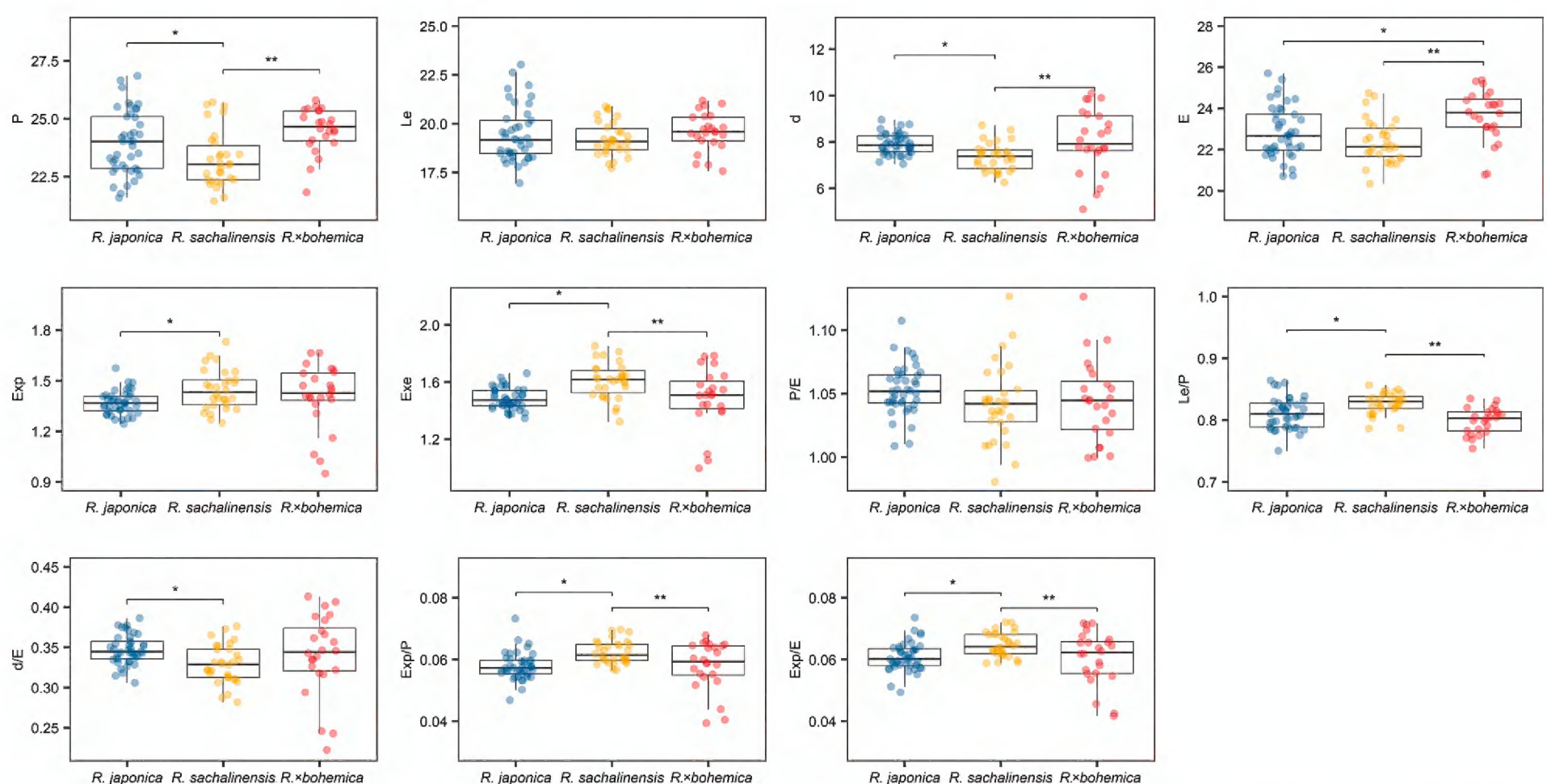


Figure 3. Boxplots of the 11 pollen grain traits analysed in the studied *Reynoutria* taxa. The points represent the mean values from each sample. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$).

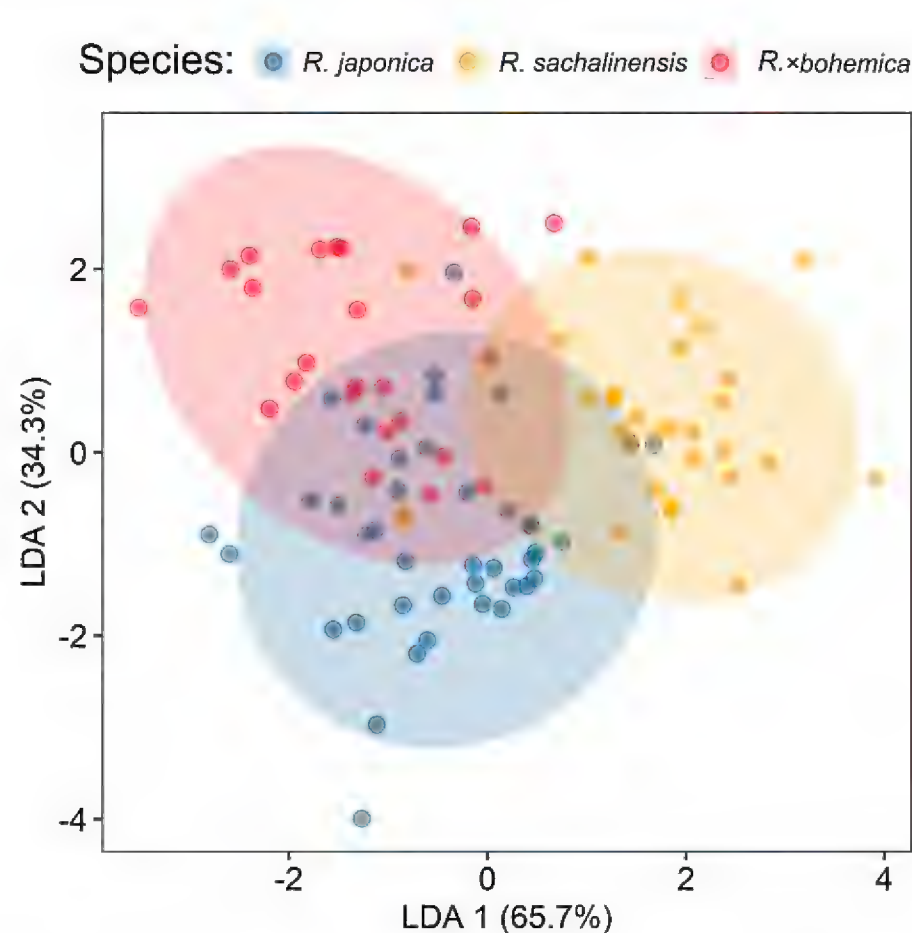


Figure 4. Biplots of linear discriminant function one (LD1) and two (LD2) for predicted variables of pollen grain traits of the studied *Reynoutria* taxa. The points represent values from each sample.

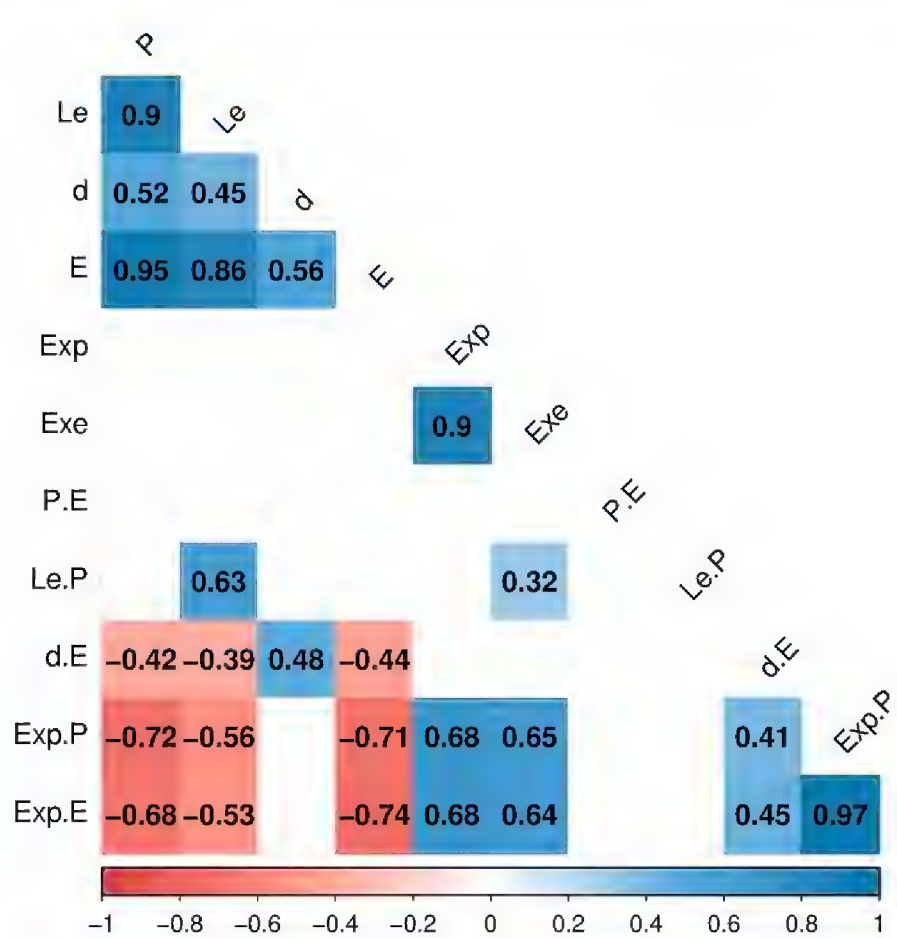


Figure 5. Heatmap for Pearson's correlation coefficients between observed traits for *R. japonica*. Significant coefficients are presented ($p < 0.05$).

Exp/E and Exp/P (0.97), as well as Exe and Exp (0.90), while strong negative correlations were observed between Exp/E and E (-0.74) and Exp/P and P (-0.72). For *R. sachalinensis*, strong significant positive correlations were found between Le and P (0.92), Exe and Exp (0.91), as well as Exp/E and Exp/P (0.89), while the most significant negative correlations were found between Le/P and P (-0.48) as well as between Le/P and P/E (-0.47). For *R. xbohemica*, strong significant positive correlations were found between d/E and d (0.95), Exp and Exe (0.96), Exp/P and Exp (0.94), as well as Exp/E and Exp/P (0.98), while the most significant negative correlations were found between P/E and E (-0.73), Le/P and P/E (-0.60), as well as P/E and Le (-0.52). The other significant correlations are presented in Figs 5–7.

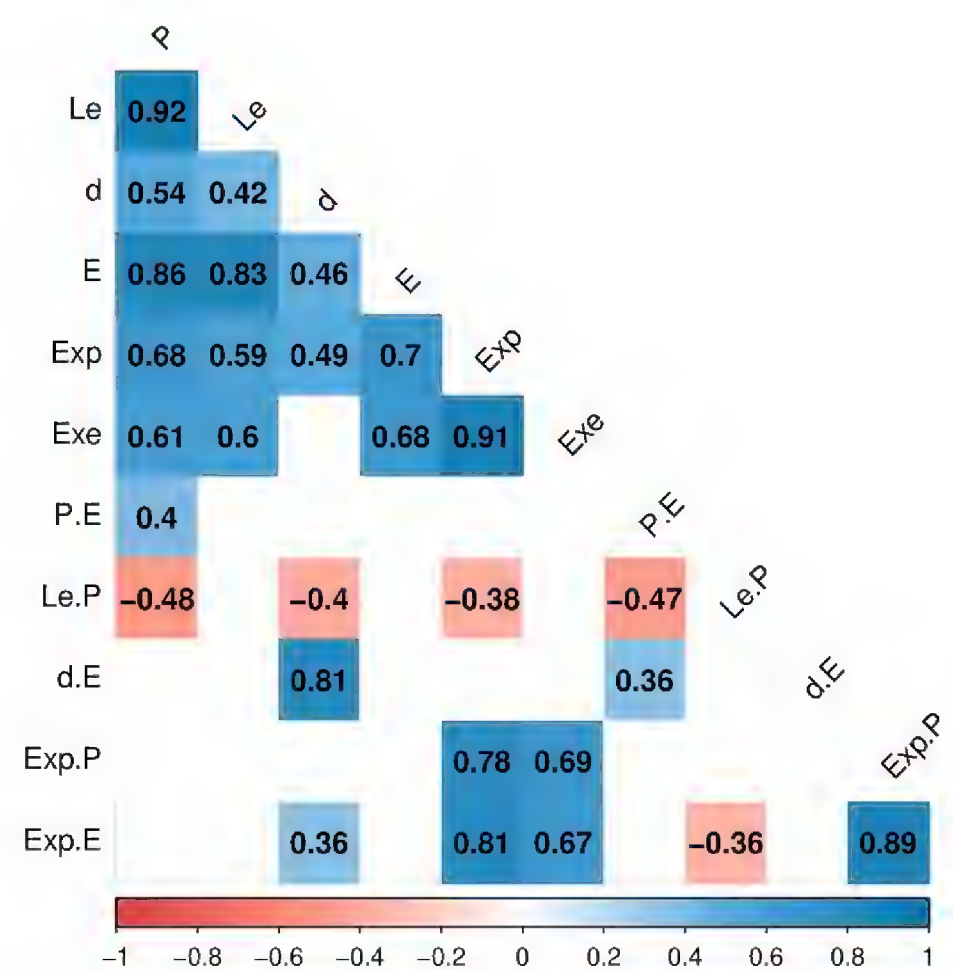


Figure 6. Heatmap for Pearson's correlation coefficients between observed traits for *R. sachalinensis*. Significant coefficients are presented ($p < 0.05$).

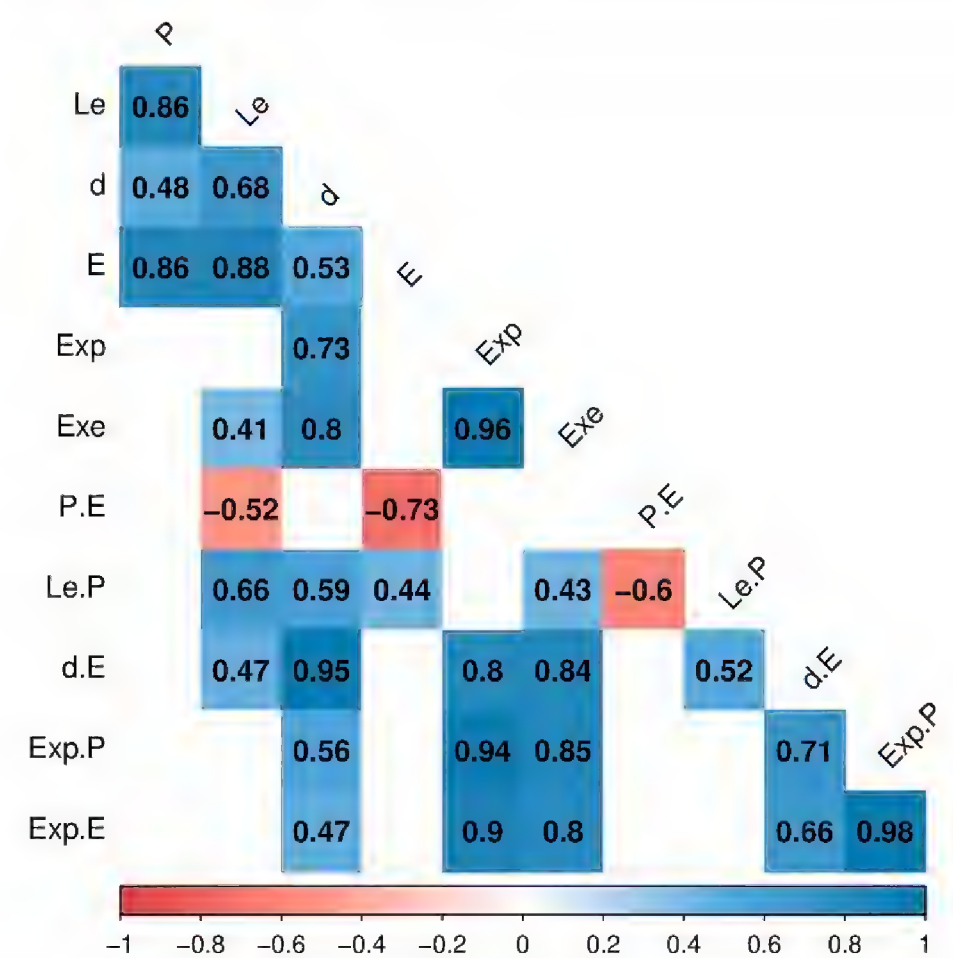


Figure 7. Heatmap for Pearson's correlation coefficients between observed traits for *R. xbohemica*. Significant coefficients are presented ($p < 0.05$).

Pollen reaction to various habitat types

The MANOVA revealed significant differences in pollen grain traits of *Reynoutria* taxa between habitat types ($F = 1.84$, Pillai = 0.70, $p = 0.011$ and $F = 6.79$, Pillai = 0.06, $p < 0.0001$, respectively, for *R. xbohemica* and *R. japonica*), except for *R. sachalinensis* ($F = 1.26$, Pillai = 0.03, $p = 0.189$).

The mixed ANOVA for *R. japonica* indicated significant differences in P, Le, E, Exe, Exp/P and Exp/E between habitats. The more conservative multiple comparison with Bonferroni correction showed significant differences between

anthropogenic habitats and floodplain forest for P, Le, E, Exp/P and Exp/E, as well as between watercourse edges and floodplain forest for Exp/P and Exp/E (Fig. 8). For *R. sachalinensis*, no significant differences in pollen traits between habitats were indicated (Fig. 9), while the results for *R. ×bohemica* indicated significant differences in P, d, Exp, Exe, d/E, Exp/P and Exp/E between habitats. The more conservative multiple comparison with Bonferroni correction showed a significant difference between anthropogenic habitats and the edges of watercourses for d, Exp, Exe, d/E, Exp/P and Exp/E traits as well as between anthropogenic habitats and floodplain forest, and between meadows and floodplain forest, for P traits (Fig. 10).

The LDA for *R. japonica* showed that pollen samples collected in mixed coniferous forest, meadows and floodplain forest were separate from those of other habitats (Fig. 11A). In *R. sachalinensis*, on the other hand, pollen grains collected from each habitat were similar to each other (Fig. 11B). In *R. ×bohemica*, samples collected in anthropogenic and watercourse-edge habitats were slightly separate from each other; separations were also visible for single points between floodplain forest and meadow habitats (Fig. 11C).

The first two LDs accounted for 79.4%, 81.2% and 89.2% of the total variability between the individual habitats, respectively, for *R. japonica*, *R. sachalinensis* and *R. ×bohemica*. For *R. japonica*, the most significant positive, linear relationship with the first LD was found for Exe (0.526), while a negative relationship was found for d (0.435). The second LD was significantly positively correlated with Le (0.401), and negatively with Exp/E (0.424). For *R. sachalinensis*, the most significant positive linear relationship with the first LD was found for Le/P (0.403), while negative relationships were found for E (0.627) and Exp (0.574). The second LD was significantly positively correlated with P/E (0.376). For *R. ×bohemica*, the most significant negative linear relationships with the first LD were found for P (-0.639) and E (-0.484).

The average values of pollen traits in *R. japonica* were analysed (Table 3). Averages for the traits P, Le and E were highest in anthropogenic habitats (P – 24.28 µm, Le – 19.71 µm, E – 23.11 µm) and lowest in the mixed coniferous forest (P – 21.59 µm, Le – 16.96 µm, E – 20.71 µm). The mean values of trait d were higher at the edges of watercourses, at 7.96 µm, and lowest for the meadow habitat, at 7.14 µm. The average values of the traits Exp and Exe were higher in the floodplain forest (Exp – 1.49 µm, Exe – 1.66 µm) and lowest in the watercourse-edge habitat (Exp – 1.35 µm, Exe – 1.46 µm). The mean values for trait P/E were the same (1.05) for all studied habitats except meadows (1.04). The value of Le/P was higher in floodplain forest (0.82) and lowest in mixed coniferous forest (0.79). The mean value of the trait d/E was higher in mixed coniferous forest (0.38) and lowest in meadow habitat (0.33), while the ratios Exp/P and Exp/E were similar in all habitats.

The mean values of the traits P, Le, d, E, Exp and Exe for *R. sachalinensis* were higher in anthropogenic habitats (P – 23.46 µm, Le – 19.33 µm, d – 7.46 µm, E – 22.60 µm, Exp – 1.46 µm, Exe – 1.62 µm) and lower in mixed broadleaved forest (P – 22.19 µm, Le – 18.48 µm, d – 6.73 µm, E – 21.64 µm, Exp – 1.34 µm, Exe – 1.50 µm) (Table 3). The described values of trait P/E were higher at the edges of watercourses (1.06) and lower in broadleaved forest (1.01). The average value of the trait Le/P was slightly higher in broadleaved forest (0.84) and lower in anthropogenic habitats (0.82). The mean values of the traits d/E, Exp/P and Exp/E for *R. sachalinensis* were similar in all habitats.

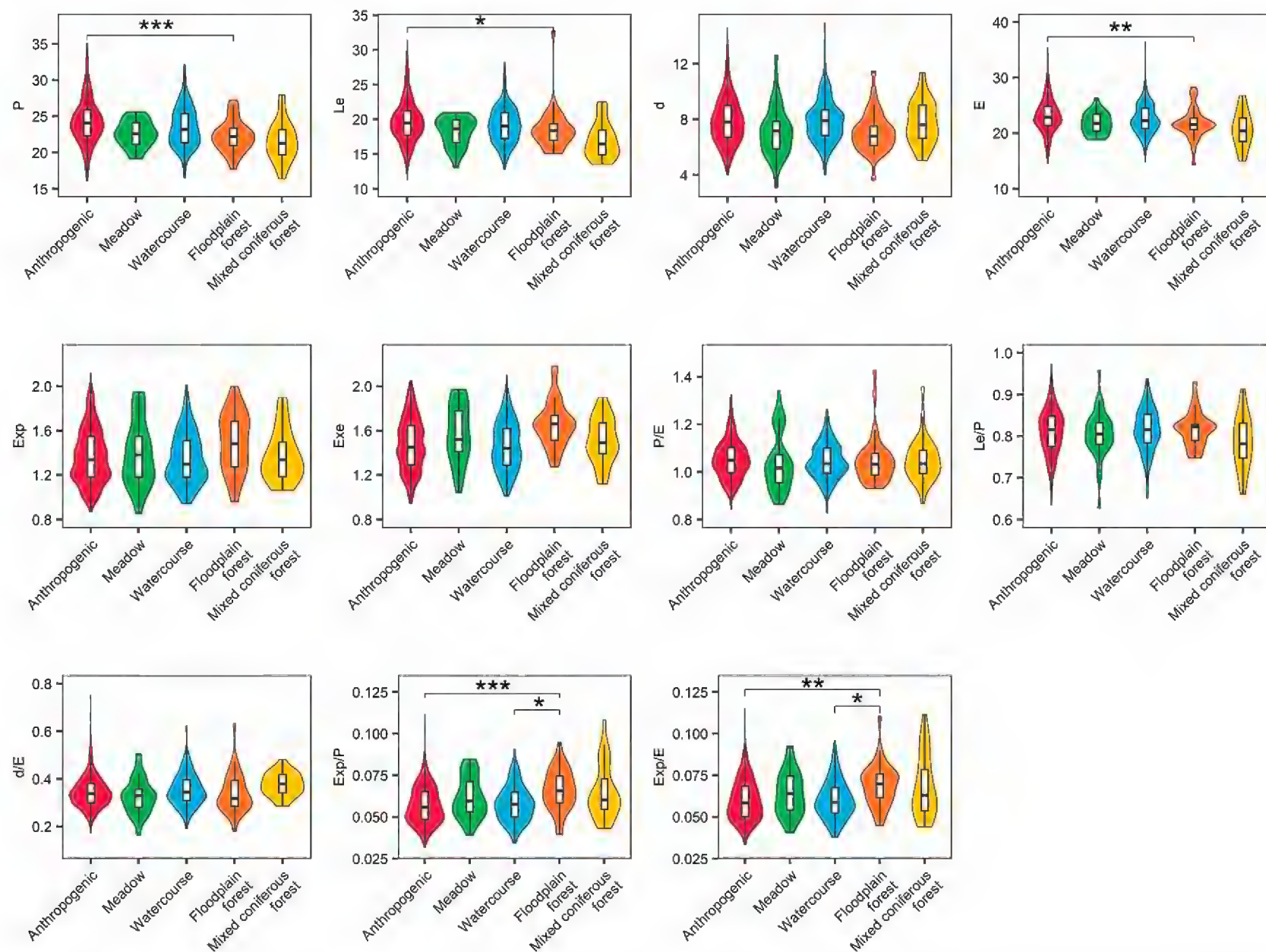


Figure 8. Boxplot and density plot of 11 pollen grain traits of *R. japonica* in relation to the habitat types studied. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

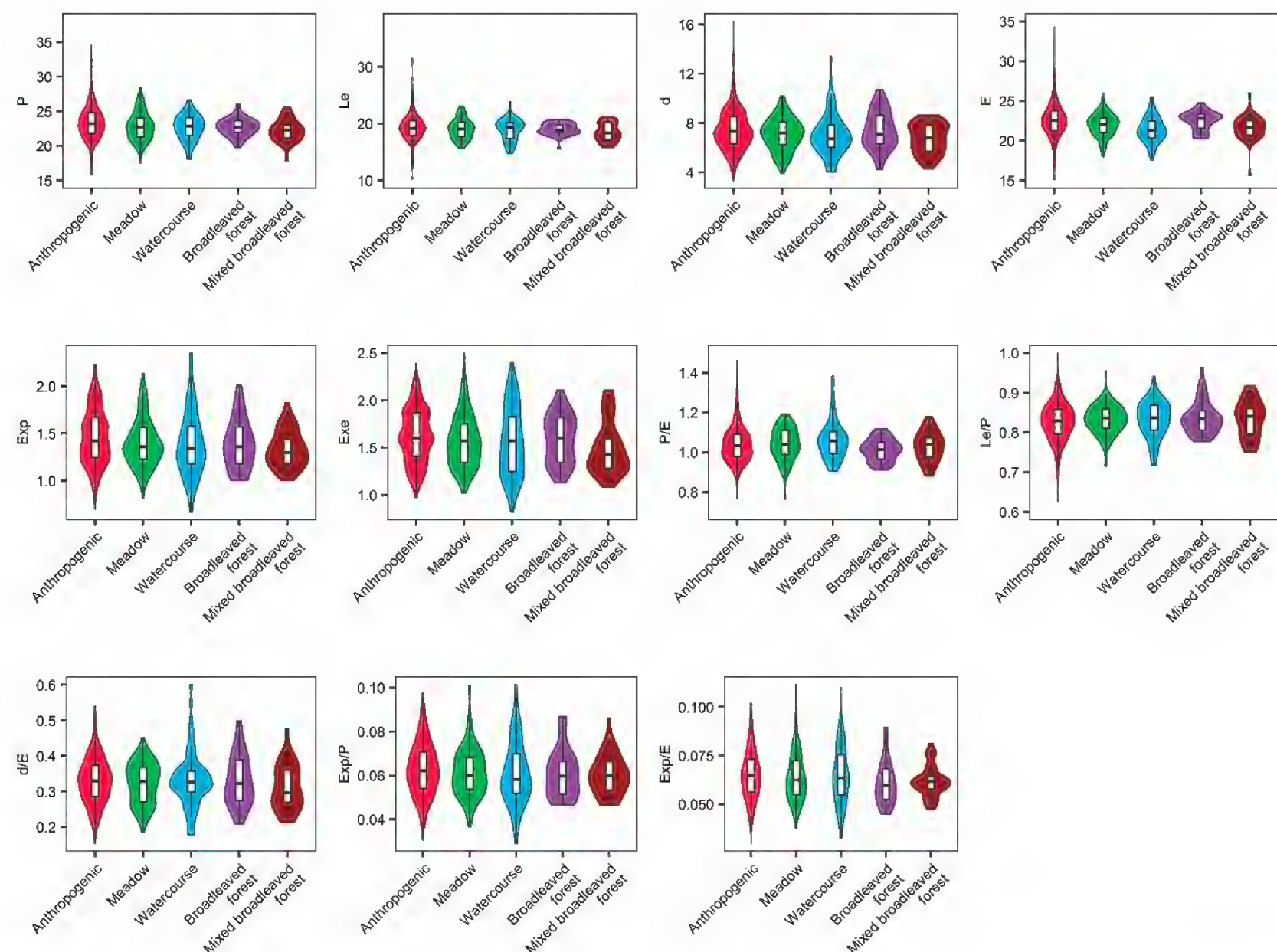


Figure 9. Boxplot and density plot of 11 pollen grain traits of *R. sachalinensis* in relation to the habitat types studied.

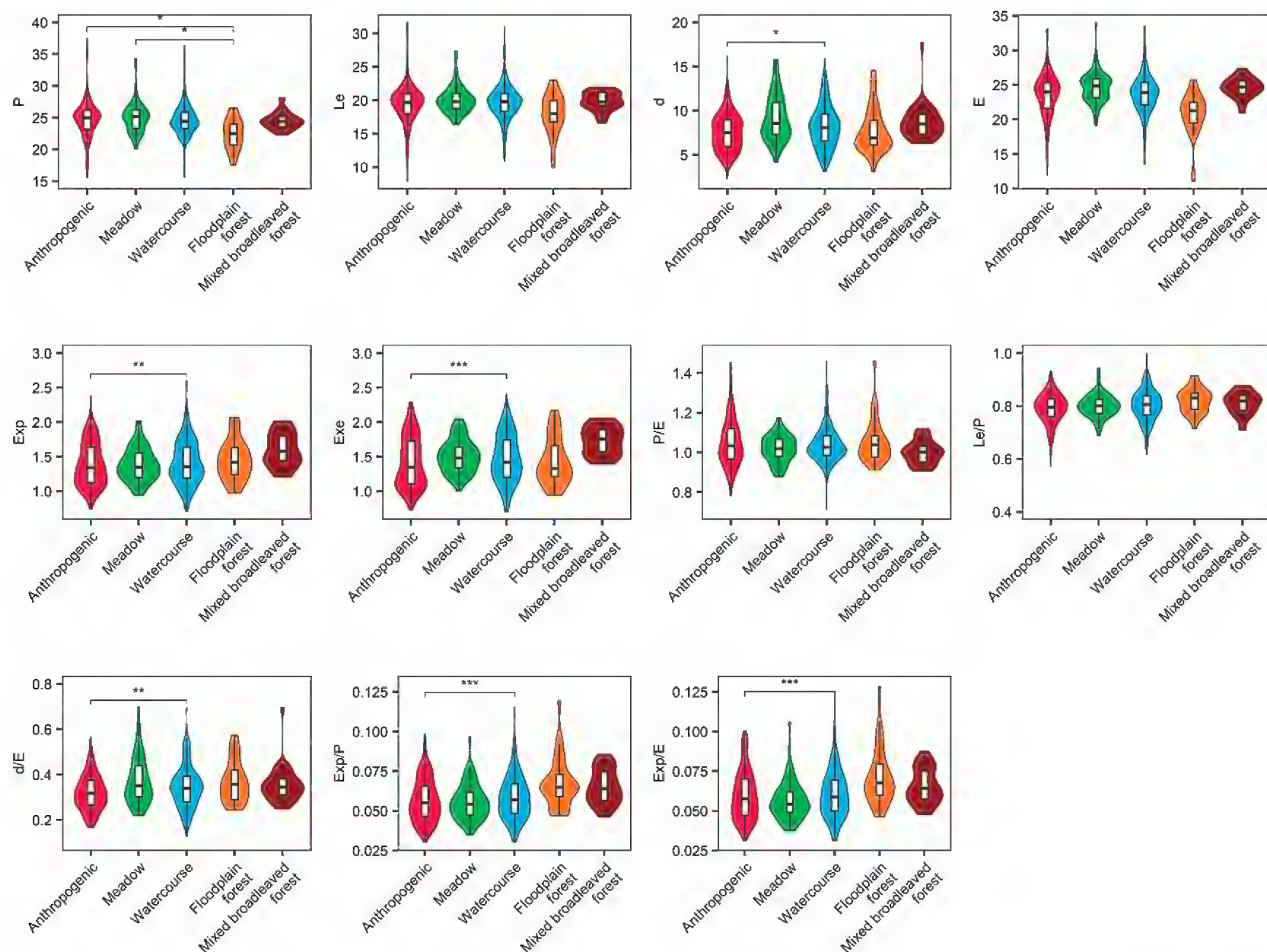


Figure 10. Boxplot and density plot of pollen grain traits of *R. xbohemica* in relation to the habitat types studied. Asterisks indicate significant differences (*P < 0.05, **P < 0.01, ***P < 0.001).

The mean values of the traits P, Le, d and E in *R. xbohemica* were higher in meadows (P – 25.09 μm , Le – 20.09 μm , d – 9.23 μm , E – 24.68 μm) and lower in floodplain forest (P – 21.82 μm , Le – 17.92 μm , E – 7.77 μm), except for trait d, which was lower in anthropogenic habitats (7.56 μm). The values of the traits Exp and Exe were higher in mixed broadleaved forest (Exp – 1.60 μm , Exe – 1.73 μm) and lower in anthropogenic habitats (Exp – 1.38 μm , Exe – 1.42 μm), while those of trait P/E were higher in anthropogenic habitats and floodplain forest (1.06), and lower in mixed broadleaved forest (1.00). The mean values of the ratios Le/P and d/E were slightly higher in floodplain forest (0.82 and 0.37, respectively) and lower in anthropogenic habitats (Le/P – 0.79, d/E – 0.32). The mean values of the traits Exp/P and Exp/E for *R. xbohemica* were similar in all habitats (Table 3).

Discussion

Reproductive strategies influence the establishment, adaptive responses and invasive potential of alien species (Rejmánek and Richardson 1996; Grotkopp et al. 2002; Barrett et al. 2008; Barrett 2011; Gioria et al. 2023). Yet, surprisingly little is known about the sexual reproduction of *Reynoutria* taxa within their invasion range. Most researchers agree that *R. sachalinensis* and *R. xbohemica* reproduce sexually very rarely (Pyšek et al. 2003; Tiébré et al. 2007; Bailey et al. 2009; Engler et al. 2011). For a long time, it was also pointed out that there is no generative reproduction in *R. japonica*, due to the lack of male-fertile plants in Europe (Bailey 1994; Beerling et al. 1994; Hollingsworth and Bailey 2000). The vast majority

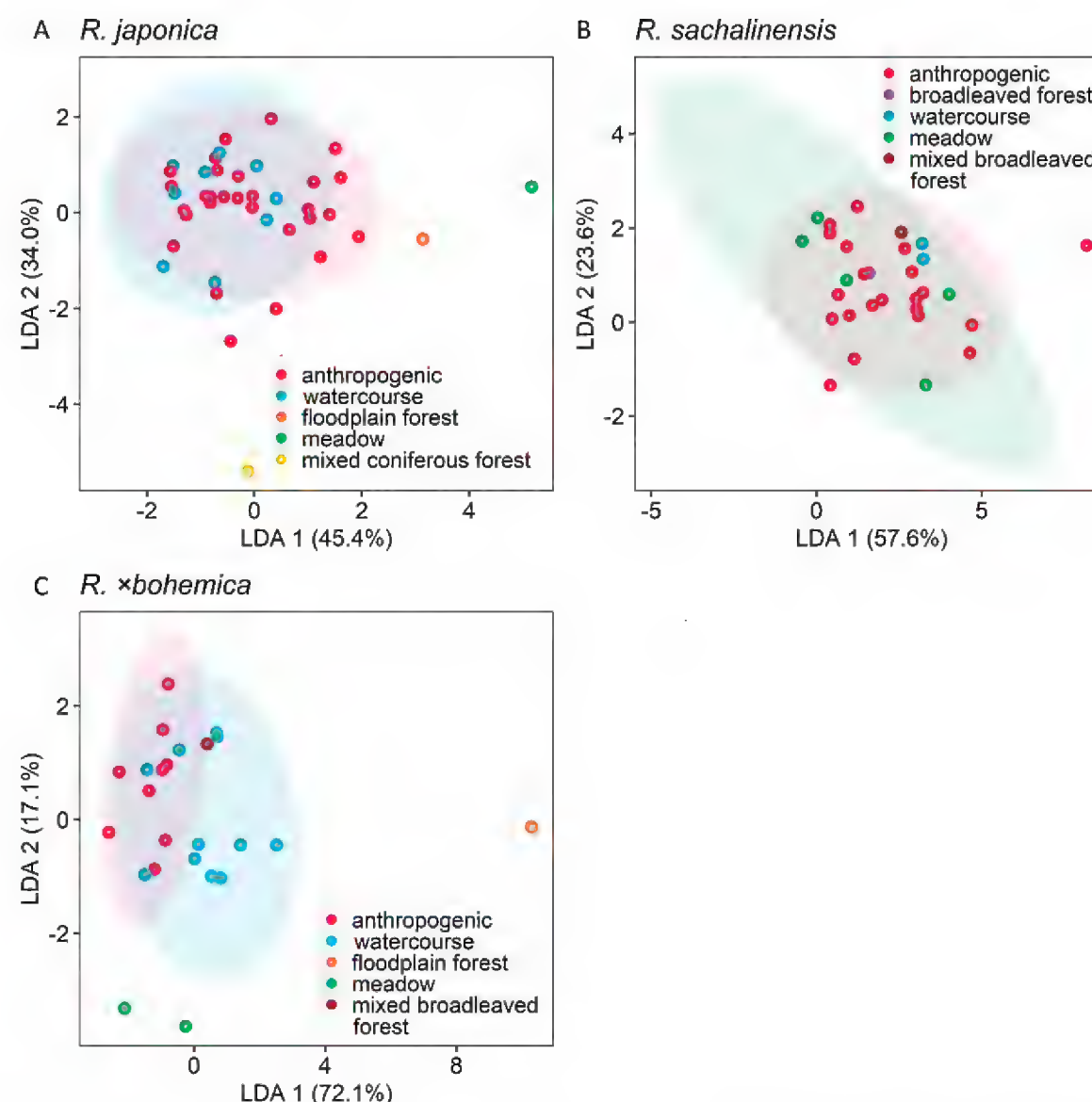


Figure 11. Biplots of linear discriminant function one (LD1) and two (LD2) for predicted variables of pollen grain traits in relation to habitat types separately for **A** *R. japonica* **B** *R. sachalinensis*, and **C** *R. xbohemica*.

of genetic studies conducted on *R. japonica* individuals from England, France, Germany and the Czech Republic have confirmed this hypothesis and shown that the species is composed of one massive female clone in Western and Central Europe (Bailey and Stace 1992; Hollingsworth et al. 1998; Hollingsworth and Bailey 2000; Mandák et al. 2005; Bailey et al. 2009; Krebs et al. 2010).

Surprisingly, different results were obtained in genetic studies of knotweed populations in another part of Central Europe (in Poland). Studies conducted on *R. japonica* showed polymorphism between individuals of the species (Bzdęga et al. 2012). The researchers concluded that this result may be the effect of multiple genetic mutations, detected at greater levels than were possible before the development of a research technique (AFLP markers) with adequate sensitivity, or resulting from several episodes of *R. japonica* introduction in this area, which could have led to the broadening range of diversity observed in the studied populations. Attention was also drawn to the fact that the detected genetic diversity of the population of *R. japonica* may have resulted from the existence of sexual reproduction of this species in Central Europe (Bzdęga et al. 2012). Studies on the genetic variability of populations of three *Reynoutria* taxa from the native and Polish invasion ranges confirmed the advantage of clonal growth over sexual reproduction in the invasion range, except for populations where the analysed taxa cohabitated. The presence of such populations containing multiple genotypes and displaying a very low level of clonality raised the possibility of sexual reproduction potentially taking place at this stand (Bzdęga et al. 2016). Research on soil seed banks conducted in Poland confirmed the germination of *Reynoutria* seeds and the development of seedlings (and then mature plants) in soil monoliths taken from 'mixed' populations, i.e. those in which individuals with two types of flowers co-occurred, or in populations composed of two or three taxa (Koszela 2013; Tokarska-Guzik et al. 2017).

Table 3. The results of the ANOVAs for pollen grain trait differences between habitats, conducted separately for all studied *Reynoutria* taxa.

<i>R. japonica</i>	F	df	P	Anthropogenic					Meadow					Watercourse					Floodplain forest					Mixed coniferous forest				
				mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV
P	4.66	4,1225	0.002	24.28	3.17	16.09	35.95	13.07	22.56	1.82	19.12	25.64	8.08	23.52	2.78	16.44	32.11	11.82	22.85	3.97	17.66	40.33	17.37	21.59	2.78	16.37	27.98	12.87
Le	4.50	4,1225	0.002	19.71	3.10	11.22	31.43	15.75	18.17	2.12	13.00	20.96	11.65	19.10	2.69	7.35	28.26	14.10	18.67	3.26	15.02	32.72	17.45	16.96	2.58	13.50	22.52	15.19
d	0.32	4,1225	0.864	7.95	1.79	4.01	17.43	22.56	7.14	1.92	3.07	12.62	26.97	7.96	1.66	4.02	14.94	20.88	7.45	2.36	3.60	17.03	31.67	7.81	1.56	5.02	11.37	19.94
E	4.03	4,1225	0.005	23.11	3.12	14.60	35.43	13.52	21.79	1.94	18.83	26.32	8.91	22.55	2.82	9.73	36.47	12.52	21.73	2.86	14.32	28.25	13.15	20.71	3.22	14.91	26.78	15.53
Exp	1.40	4,1225	0.241	1.37	0.25	0.87	2.12	17.90	1.39	0.29	0.85	1.95	20.94	1.35	0.23	0.94	2.01	16.95	1.49	0.27	0.96	2.00	17.87	1.37	0.23	1.06	1.90	16.92
Exe	3.61	4,1225	0.009	1.48	0.24	0.94	2.05	16.03	1.58	0.26	1.04	1.97	16.76	1.46	0.22	1.01	2.10	15.09	1.66	0.21	1.27	2.18	12.72	1.51	0.21	1.12	1.90	13.92
P/E	0.21	4,1225	0.934	1.05	0.09	0.69	1.77	8.34	1.04	0.12	0.86	1.34	11.80	1.05	0.11	0.71	2.38	10.70	1.05	0.11	0.93	1.43	10.18	1.05	0.09	0.87	1.36	8.98
Le/P	0.49	4,1225	0.739	0.81	0.06	0.60	1.00	7.31	0.80	0.06	0.63	0.96	7.57	0.81	0.06	0.34	0.94	7.86	0.82	0.04	0.75	0.93	5.17	0.79	0.06	0.66	0.91	8.18
d/E	1.63	4,1225	0.177	0.34	0.07	0.17	0.75	19.38	0.33	0.08	0.16	0.51	23.35	0.36	0.08	0.19	0.89	21.29	0.34	0.09	0.18	0.63	25.14	0.38	0.05	0.29	0.48	12.83
Exp/P	5.30	4,1225	0.001	0.06	0.01	0.03	0.11	20.69	0.06	0.01	0.04	0.08	20.05	0.06	0.01	0.03	0.09	18.75	0.07	0.01	0.04	0.09	18.89	0.07	0.02	0.04	0.11	24.87
Exp/E	4.66	4,1225	0.002	0.06	0.01	0.03	0.11	21.24	0.06	0.01	0.04	0.09	20.51	0.06	0.01	0.04	0.16	21.55	0.07	0.01	0.04	0.11	19.04	0.07	0.02	0.04	0.11	27.39
<i>R. sachalinensis</i>	F	df	P	Anthropogenic					Meadow					Watercourse					Broadleaved forest					Mixed broadleaved forest				
				mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV
P	0.71	4,895	0.591	23.46	2.79	14.96	44.15	11.88	22.89	2.10	17.48	28.44	9.17	22.64	1.86	18.03	26.67	8.23	22.71	1.33	19.72	26.06	5.86	22.19	1.69	17.76	25.59	7.62
Le	0.57	4,895	0.684	19.33	2.51	10.18	39.15	12.97	19.04	1.78	15.58	23.09	9.35	18.87	1.88	14.67	23.84	9.96	19.03	1.12	15.48	20.78	5.91	18.48	1.60	15.77	21.19	8.64
d	0.79	4,895	0.545	7.46	1.69	3.34	16.26	22.70	7.09	1.34	3.92	10.23	18.94	7.08	1.78	4.02	13.41	25.10	7.44	1.57	4.20	10.76	21.13	6.73	1.22	4.29	8.66	18.11
E	1.16	4,895	0.354	22.60	2.59	10.97	34.32	11.48	21.98	1.50	17.98	25.98	6.84	21.41	1.65	17.56	25.52	7.71	22.48	1.24	20.20	24.80	5.54	21.64	1.71	15.59	26.04	7.92
Exp	0.73	4,895	0.583	1.46	0.29	0.70	2.23	20.08	1.40	0.26	0.82	2.14	18.24	1.39	0.35	0.67	2.35	25.18	1.39	0.27	1.00	2.01	19.32	1.34	0.20	1.00	1.83	15.11
Exe	0.51	4,895	0.731	1.62	0.29	0.97	2.39	17.76	1.57	0.29	1.02	2.50	18.36	1.56	0.37	0.82	2.40	23.60	1.59	0.26	1.13	2.11	16.24	1.50	0.28	1.08	2.11	18.81
P/E	0.74	4,895	0.576	1.04	0.10	0.77	1.88	9.34	1.04	0.08	0.76	1.19	7.77	1.06	0.10	0.90	1.39	9.26	1.01	0.06	0.91	1.12	5.59	1.03	0.08	0.88	1.18	7.46
Le/P	0.28	4,895	0.883	0.82	0.06	0.43	1.00	6.70	0.83	0.04	0.71	0.95	4.42	0.83	0.05	0.72	0.94	5.59	0.84	0.04	0.78	0.96	5.24	0.83	0.05	0.75	0.92	5.71
d/E	0.19	4,895	0.939	0.33	0.07	0.15	0.54	19.99	0.32	0.06	0.18	0.45	18.30	0.33	0.08	0.18	0.60	24.25	0.33	0.07	0.21	0.50	22.34	0.31	0.06	0.21	0.48	20.58
Exp/P	0.28	4,895	0.889	0.06	0.01	0.03	0.10	19.48	0.06	0.01	0.04	0.10	18.38	0.06	0.01	0.03	0.10	24.03	0.06	0.01	0.05	0.09	18.37	0.06	0.01	0.05	0.09	15.51
Exp/E	0.36	4,895	0.837	0.07	0.01	0.03	0.10	19.96	0.06	0.01	0.04	0.11	19.86	0.06	0.02	0.03	0.11	23.55	0.06	0.01	0.04	0.09	19.84	0.06	0.01	0.05	0.08	13.40
<i>R. xobohenica</i>	F	df	P	Anthropogenic					Meadow					Watercourse					Floodplain forest					Mixed broadleaved forest				
				mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV	mean	SD	min	max	CV
P	3.04	4, 715	0.042	24.64	3.33	13.56	37.50	13.53	25.09	2.53	20.05	34.28	10.09	24.55	2.76	13.42	36.35	11.25	21.82	3.29	11.90	26.53	15.10	24.56	1.43	22.24	28.17	5.83
Le	0.84	4, 715	0.516	19.46	3.05	7.90	31.65	15.69	20.09	2.09	16.39	27.35	10.42	19.72	2.66	10.87	30.98	13.48	17.92	3.09	9.92	23.02	17.23	19.86	1.43	16.55	21.99	7.22
d	2.87	4, 715	0.046	7.56	2.14	2.23	16.17	28.29	9.23	2.68	4.18	15.79	29.07	8.21	2.45	3.09	15.86	29.88	7.77	2.60	3.04	14.56	33.48	8.76	2.13	6.30	17.73	24.31
E	2.11	4, 715	0.115	23.53	3.25	11.94	33.15	13.82	24.68	2.33	19.08	34.05	9.43	23.68	2.84	13.52	33.53	12.01	20.78	3.24	11.06	25.76	15.61	24.59	1.50	20.89	27.44	6.10
Exp	5.06	4, 715	0.004	1.38	0.32	0.67	2.38	23.16	1.39	0.24	0.94	2.02	17.63	1.41	0.32	0.63	2.60	22.87	1.46	0.29	0.97	2.07	20.12	1.60	0.24	1.20	2.02	15.30
Exe	11.52	4, 715	<0.0001	1.42	0.37	0.60	2.29	26.44	1.51	0.24	1.00	2.05	16.17	1.46	0.36	0.48	2.40	24.51	1.43	0.34	0.94	2.17	23.43	1.73	0.20	1.39	2.06	11.80
P/E	1.14	4, 715	0.368	1.06	0.13	0.78	1.74	12.50	1.02	0.07	0.88	1.17	6.64	1.04	0.10	0.71	1.63	9.81	1.06	0.11	0.91	1.46	10.86	1.00	0.06	0.90	1.12	5.96
Le/P	1.38	4, 715	0.276	0.79	0.06	0.38	0.93	7.99	0.80	0.04	0.69	0.94	5.56	0.80	0.06	0.62	1.00	7.77	0.82	0.05	0.71	0.91	5.74	0.81	0.04	0.71	0.88	5.49
d/E	4.16	4, 715	0.011	0.32	0.08	0.17	0.56	24.75	0.37	0.10	0.22	0.70	27.97	0.35	0.09	0.13	0.70	26.76	0.37	0.09	0.24	0.57	24.72	0.36	0.08	0.25	0.70	23.13
Exp/P	5.25	4, 715	0.004	0.06	0.01	0.03	0.10	24.28	0.06	0.01	0.03	0.10	20.63	0.06	0.01	0.02	0.12	24.61	0.07	0.02	0.05	0.12	23.76	0.07	0.01	0.05	0.09	16.81
Exp/E	4.9	4, 715	0.005	0.06	0.02	0.03	0.10	26.09	0.06	0.01	0.04	0.11	21.98	0.06	0.02	0.03	0.12	25.07	0.07	0.02	0.05	0.13	25.34	0.07	0.01	0.05	0.09	16.95

In Europe, knotweed seedlings in the wild were recorded e.g. in Slovenia, Belgium, Germany and Poland (Tiébré et al. 2007; Engler et al. 2011; Funkenberg et al. 2012; Strgulc Krajšek and Dolenc Koce 2015; Tokarska-Guzik et al. 2017; Bzdęga et al. 2022), and in North America in the United States and Canada (Forman and Kesseli 2003; Groeneveld et al. 2014), but the mortality of seedlings tested in the field experiment was very high (Kadlecová et al. 2024). This, however, seems to be the result of unfavourable environmental conditions during the germination (e.g. water stress) rather than an inappropriate genetic or cytological make-up (Meredá et al. 2023; Kadlecová et al. 2024). In our research, we found that all three analysed taxa of the genus *Reynoutria* – including the commonly considered male-sterile *R. japonica* – produce pollen, which positively validates our first hypothesis. Moreover, male-fertile individuals of *R. japonica*, as in the populations of this species studied in North America (Forman and Kesseli 2003), do not appear to be an anomaly, as pollen grains were detected in the flowers of all examined *R. japonica* specimens. However, only in about 10% of them was the amount of pollen large – comparable to its amount in the heavily pollen-producing specimens of *R. sachalinensis* and *R. ×bohemica*.

Single male-fertile plants have already been observed in invasion populations of *R. japonica* in the United States, where at least one male-fertile plant was found to occur within the pollination distance of each female plant (Forman and Kesseli 2003); the same phenomenon was observed in Germany (Child and Wade 2000). Still, as Alberternst and Böhmer (2011) emphasise, this phenomenon seemed to be very rare. Our results indicating the occurrence of male-fertile individuals of *R. japonica* in Central Europe, including single individuals providing a large supply of pollen, are in line with these observations, but also clearly correspond to the results of genetic studies of the *R. japonica* in this area (Bzdęga et al. 2012, 2016). This also seems to indicate that the source of *R. japonica* polymorphism in Central Europe may be isolated cases of sexual reproduction in this species. Although the production of pollen itself does not determine, for example, its viability and germination (Dafni and Firmage 2000) or the possibility of effective pollen transfer and pollination (Waytes et al. 2022), the presence of fertile males certainly promotes effective sexual reproduction in the case of this species. Therefore, in such situations, as noted by Grimsby et al. (2007), more attention should be paid to the survival of *R. japonica* seeds and seedlings. Although the recruitment of knotweed seedlings in Europe is strongly dependent on climatic conditions (Beerling et al. 1994; Bailey et al. 2009; Kadlecová et al. 2024), which cause most seeds to rot in the ground (Bailey et al. 2009; Koszela 2013), it is known that an increase in the frequency of sexual reproduction is responsible for the increased ability of plants to adapt to environmental stochasticity (Lei 2010). This in turn can significantly increase invasiveness, which should be taken into account during the monitoring of *R. japonica* and other invasive species. As noted by Soll (2004), if extensive sexual reproduction in *R. japonica* is confirmed in natural conditions, it would be necessary to change the planning strategy for population control of this species at the landscape level.

Knowledge of the biology of the species, including its method of reproduction and spread, is crucial in developing effective methods to limit the phenomenon, given the increasing negative impact of invasive alien species on biodiversity, the economy and human quality of life (IPBES 2023). Taxa of the *Reynoutria* genus are considered as posing a significant environmental threat and are particularly difficult to eliminate, mainly due to their high ability to regenerate from underground rhizomes (even small fragments) and above-ground shoots (e.g. Aguilera

et al. 2010; Alberternst and Böhmer 2011; Tokarska-Guzik et al. 2017; Švec et al. 2024). An increase in the share of sexual reproduction in the secondary range of these taxa may affect the effects of remedial actions taken. So far, dozens of control methods have been described for knotweed, some of which are still being tested (Dusz et al. 2021; Bzdęga et al. 2022, 2024, Švec et al. 2024). The descriptions of control methods quite rarely indicated the need to take into account sexual reproduction, pointing primarily to the possibility of seedlings appearing in 'mixed' populations, i.e. composed of two or three taxa. Our results regarding the possibility of sexual reproduction in *R. japonica* populations suggest the need to take this aspect into account when planning control methods for all knotweed populations. For example, the proposed long-term strategy for combating knotweed (Švec et al. 2024) should take into account actions that prevent knotweed from blooming/seed setting, controlling the possibility of seedlings appearing in gaps in dense knotweed canopies as a result of plant elimination measures, or planning the treatment of soil (in the event of its possible movement) which may potentially, in addition to the rhizomes, also contain viable knotweed seeds.

There are only a few publications regarding the pollen morphology of the genus *Fallopia* and *Reynoutria*. In the palynological studies carried out to date on species from the family Polygonaceae, representatives of this genus were usually not included (e.g. Nowicke and Skvarla 1977; Zhong-ze et al. 1999; Hong et al. 2005; Mosafari and Keshavarzi 2011; Raycheva et al. 2021). According to Nowicke and Skvarla (1977) and Faegri et al. (2000), Polygonaceae species are very diverse in terms of their pollen features, especially in exine structure and ornamentation. The research of the above-cited authors and our results also confirm this assumption concerning the *Fallopia* and *Reynoutria* genus. The most important pollen features of this genus are the exine ornamentation type, polar-axis length (P) and pollen shape (P/E ratio). According to Hong et al. (2005), some species of *Fallopia* (e.g. *F. convolvulus*, *F. dumetorum*) show a dimorphic exine (smooth in the mesocolpium area, but punctate in the region of the colpi), as was earlier observed by Nowicke and Skvarla (1977). However, the described dimorphism did not occur in the taxa we examined.

To date, the pollen morphology of five *Fallopia* and *Reynoutria* taxa has been examined: *F. convolvulus* (Yasmin et al. 2009; Halbritter et al. 2018; Heigl 2020; Paul and Chowdhury 2020), *F. dumetorum* (Yasmin et al. 2009) and *F. aubertii*, *F. baldschuanica* and *F. japonica* (*R. japonica*) (Halbritter et al. 2018; Heigl 2020, 2021). The results of the present study and of the few studies cited above agree that the pollen grains of the genus are isopolar, tricolporate monads. According to palynologists, the pollen sizes are small (P = 21.00 µm in *F. convolvulus* and *F. dumetorum*; Yasmin et al. 2009) or, also in *F. convolvulus* (Paul and Chowdhury 2020), small or medium-sized (Halbritter 1998; Heigl 2020, 2021). Our research found both sizes of pollen, with a clear advantage of small pollen (70%) over medium-sized pollen (30%); additionally, the mean value of P was higher than those of the cited researchers, at 23.88 µm. These differences are probably due to the fact that we studied species other than those of the above-cited authors, but also to the very large sample of pollen grains measured in our study. The pollen shape (P/E ratio) described in other *Fallopia/Reynoutria* species was subprolate, sometimes prolate spheroidal (Yasmin et al. 2009), prolate spheroidal (Paul and Chowdhury 2020) or usually spheroidal according to Halbritter (1998) and Heigl (2020, 2021). Our results showed seven pollen shape types (mostly prolate spherical and oblate spherical, rarely subprolate, suboblate, prolate, oblate or perprolate). In the study of

Yasmin et al. (2009), the mean length of the ectocolpus (Le) was 12.0–12.8 μm ; a similar result (12.11 μm) was obtained by Paul and Chowdhury (2020), while Heigl (2020) did not provide values for this feature. In our study, the average value of this feature was 19.41 μm . The exine thickness ranged from 2.00 μm in *F. dumetorum* to 3.8 μm in *F. convolvulus* (Yasmin et al. 2009), while our study found it to be 1.46 (0.48–2.6) μm in *Reynoutria* taxa. Based on SEM observations of exine ornamentation, Halbritter (1998) described that of *F. convolvulus* as psilate, microechinate and perforate, and that of *F. baldschuanica* as perforate, microechinate and microgemmate. On the other hand, Yasmin et al. (2009) distinguished two pollen types: an *F. convolvulus* type (microechinate-punctate) and *F. dumetorum* type (reticulate). Paul and Chowdhury (2020) reported the same exine type in *F. convolvulus* (microechinate-punctate). Here, we found that the exine ornamentation under SEM appeared rugulate and fossulate perforate in *R. sachalinensis* and reticulate perforate in *R. japonica* and *R. \times bohemica*. These are different types to those described above because the exine of these taxa has not been examined to date. This result confirms the opinion of Nowicke and Skvarla (1977) and Faegri et al. (2000) about the large diversity of exine ornamentation types in the genus *Fallopia* and *Reynoutria*.

All studied *Reynoutria* taxa are now widely naturalised in Europe and pose a threat to native ecosystems (Tokarska-Guzik et al. 2006; Gerber et al. 2008; Moravcová et al. 2011; Chmura et al. 2015), but understanding the conditions of their invasion in different habitats and the causes of these differences requires taking into account many factors (Bímová et al. 2004). Generally, research has shown that in Europe, three knotweed taxa invade similar habitats, although certain habitat preferences have been observed for each of them (Bímová et al. 2004; Tiébré et al. 2008; Tokarska-Guzik et al. 2017). Habitat patch colonisation dynamics and distribution patterns analysed at landscape scale showed that *R. japonica* seems to have a wide ecological range (Mandák et al. 2004; Tiébré et al. 2008; Aguilera et al. 2010) – according to Bímová et al. (2004), the widest among the three knotweed taxa. The species invades both anthropogenic and natural sites. It grows on roadsides, railway embankments, urban and post-industrial wastelands, parks, cemeteries and home gardens, but also invades natural sites (river banks, forest edges and thickets and forests, especially riparian forests) (Bímová et al. 2004; Mandák et al. 2004; Tokarska-Guzik et al. 2017). *R. sachalinensis* is rarer than *R. japonica* in the invasion range. The species is most often recorded in sites more or less influenced by human activity, like urban or disturbed areas (gardens, parks, railsides and roadsides), but sometimes also colonises riverbanks (Shaw 2008; Tokarska-Guzik et al. 2009, 2017; Chmura et al. 2015). The habitat requirements of *R. \times bohemica* are similar to those of the parental species, but Bímová et al. (2004) and Tiébré et al. (2008) showed particularly similar patterns of habitat selection between *R. japonica* and *R. \times bohemica*, and Bímová et al. (2004) indicated that in the Czech Republic, *R. \times bohemica* mainly occupies riverside habitats.

Our research found that, although pollen grains are considered the most conservative plant organs (Stace 1989), habitat conditions influence some pollen quantitative characteristics of *R. japonica* and *R. \times bohemica*. For *R. sachalinensis*, no significant differences in pollen traits between habitats were indicated; thus, our second hypothesis that habitat conditions affect the morphological structure of all knotweed pollen should be partially rejected. For *R. japonica*, differences in pollen characteristics between habitats were found in P, Le, E, Exe, Exp/P and Exp/E,

and for *R. ×bohemica* in P, d, Exp, Exe, d/E, Exp/P and Exp/E. This indicates that P, Exp, E, Exp/P and Exp/E can be considered the knotweed pollen traits most strongly responsive to habitat conditions, which partially coincides with rarely obtained research results for other plant species. Wrońska-Pilarek et al. (2023) showed that in *Convallaria majalis*, Ex/LA and Ex are the pollen traits that respond most strongly to habitat conditions. It is therefore possible that exine thickness is a pollen trait that is sensitive to habitat properties, which is particularly important because the success of pollen-tube penetration depends on exine thickness in the colpus membrane (Wang and Dobritsa 2018); this means that the properties of the habitat may affect the fertilisation process.

These results also indicate that habitat conditions similarly affect the quantitative features of the morphological structure of *R. japonica* and *R. ×bohemica* pollen. These observations correspond to the results of the cluster analysis of genetic polymorphism between pairs of taxa, which showed that *R. ×bohemica* is much more similar to *R. japonica* than to *R. sachalinensis* (Bzdęga et al. 2012, 2016), a fact that may translate into the similar reaction of these taxa to diverse environmental conditions.

We obtained similar results by examining inter- and intraspecific variability of knotweed pollen growing in different habitats. We found that the pollen of *R. sachalinensis* collected from different habitats were similar, whereas there was some variability of pollen from *R. japonica* and *R. ×bohemica* growing in different conditions. In *R. ×bohemica*, differences were found between pollen samples collected from anthropogenic habitats and the edges of watercourses. Since, as noted by Bímová et al. (2004), the phenotypic variability of *R. ×bohemica* is greater than the variability of both parental taxa, perhaps this variability also translates into other, previously undescribed morphological features of this plant and affects the stronger reaction of this taxon's pollen to habitat conditions. In the case of both *R. japonica* and *R. ×bohemica*, individual plants growing in forests (including riparian forests, among other types) and in abandoned meadows produced different pollen from the other examined individuals. In Central Europe, the habitats most invaded by *Reynoutria* taxa are more often flooded than the other habitat types (Bímová et al. 2004), and in Belgium, Tiébré et al. (2008) demonstrated the importance of aquatic and humid habitats and linear landscape structures in the distribution patterns of *R. japonica* and *R. ×bohemica*, which show high genetic similarity in such conditions (Bzdęga et al. 2012). It can therefore be assumed that individuals growing in wet habitats, such as the periodically flooded edges of watercourses, riparian forests or, for example, abandoned wet meadows, produce slightly different pollen to those growing in anthropogenic habitats. A detailed analysis of the average values of pollen traits showed that values of the P, Le and E for *R. japonica* and *R. sachalinensis* were highest in anthropogenic habitats, and for *R. ×bohemica* in abandoned meadows. We also found that for all examined taxa, the average values of these features were lowest in the forests. This means that *R. japonica* and *R. sachalinensis* develop the largest pollen in anthropogenic habitats, and *R. ×bohemica* in abandoned meadows, while the smallest pollen grains are formed by knotweeds growing in forests. This may be related to the fact that pollen grains under more intense desiccation stress during flowering periods tend to be larger (Ejsmond et al. 2011). It is therefore possible that in open areas (like anthropogenic habitats or meadows), where the substrate dries out faster, the desiccation stress is greater than in more stable forest communities, affecting the size of knotweed grains.

In light of the very complex habitat–plant relationships and, to our knowledge, the lack of a study linking pollen morphology with natural environmental factors, we were aware that the interpretation of the obtained results regarding the effect of habitat on pollen would be difficult. Another limitation of our study is the imbalance in the number of samples collected from each habitat type. Sample sizes were determined based on the natural distribution and abundance of *Reynoutria* taxa in the study area, and this imbalance reflects the actual occurrence of taxa in different habitats. Although this sampling design is ecologically relevant, it may introduce some statistical limitations, especially when comparing pollen traits across habitat types. Furthermore, the diversity of forest habitats in terms of environmental variables such as fertility and moisture may contribute to the variability of pollen traits. These factors may influence the results of the analyses, especially in habitats where environmental conditions are significantly different. This introduces another source of variability that should be considered when interpreting the results. We acknowledge this as a limitation and suggest that future studies could include a more controlled sampling of habitat variables to better isolate the effects of environmental factors on pollen traits. However, because the gametophyte stage is a key stage in the plant life cycle, it seems important to try to understand how environmental fluctuations affect its fitness (Matamoro-Vidal et al. 2016). Our studies indicate that certain morphological pollen traits of knotweeds are influenced by habitat conditions. Since we often assume that morphological traits are closely related to the functions they perform and vice versa (e.g. Lauder 1981), it seems that the focus of further research should be to determine whether changes in the morphological structure of pollen induced by habitat conditions affect its viability. This is especially important, because our study clearly indicates that the gametophytic phase of the plant life cycle of *Reynoutria* species may play an increasingly important role in the invasion process of these species, and that this may be partly determined by habitat conditions.

Conclusions

- The most important novel result of our research is demonstrating that in the studied knotweed populations in Central Europe, *R. japonica*, *R. sachalinensis* and *R. ×bohemica* produce pollen, although it was hitherto thought that in the invasion range, plants identified as *R. japonica* are male sterile.
- A clear difference was found in the amount of pollen among the examined taxa. The amount of *R. japonica* pollen was lower than that of the other two studied taxa, but approximately 10% of individuals of this species had abundant pollen. Further investigations are needed to determine what role, if any, sexual reproduction plays in the spreading system and evolution of *R. japonica* in Central Europe.
- Based on pollen morphological features, it was possible to distinguish *R. sachalinensis*, which has different exine ornamentation (rugulate, fossulate perforate), from the other two taxa (reticulate perforate).
- The following pollen traits showed the most pronounced response to different habitat conditions in the seven studied habitats: P, Exp, E, Exp/P and Exp/E. Of the three taxa, the hybrid *R. ×bohemica* showed the strongest response to habitat conditions.

- The greatest interspecific variability was found between *R. japonica* and *R. sachalinensis*, and the smallest between *R. japonica* and *R. ×bohemica*. The greatest intraspecific variability was demonstrated by *R. ×bohemica*, and the smallest by *R. sachalinensis*.
- We found that the reaction of pollen grains collected from seven different habitat types varied. Pollen grains of *R. sachalinensis* were more or less similar. In *R. japonica*, pollen samples collected in mixed coniferous forest, meadows and floodplain forest were separate from those of other habitats, and in *R. ×bohemica*, samples from anthropogenic and watercourse-edge habitats were slightly separate from each other; separations were also visible for single points between floodplain forest and meadow habitats. The reaction was not always clearly visible, probably because pollen grains, as the most conservative plant organs, respond slowly to changing habitat conditions.
- In our opinion, the finding of male-fertile *R. japonica* individuals in Central Europe has important implications for the management of knotweed in Europe and highlights the importance of regional research in understanding the biology of invasive plant species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization B.W. and D.W.-P.; Methodology B.W., D.W.-P. and Ł.D.; Software Ł.D., B.W. and D.W.-P.; Validation B.W. and D.W.-P.; Formal analysis Ł.D., B.W., D.W.-P.; Investigation B.W., D.W.-P., K.B., B.T.-G. and Ł.D.; Data curation B.W., K.B., B.T.-G., Z.D., A.G.; Writing—original draft preparation B.W., D.W.-P., K.B., Ł.D.; Writing—review and editing B.W., D.W.-P., K.B., B.T.-G., Z.D.; Visualization Ł.D., B.W.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Appendix 1

Table A1. List of research sites of *R. japonica*, *R. sachalinensis* and *R. ×bohemica* located in Poland.

Sample No.	Species	Localities	Geographical coordinates	Collector
1.1	<i>R. japonica</i>	Poland, Kaniów	49.954841°N, 19.055690°E	B. Tokarska-Guzik
1.2	<i>R. japonica</i>	Poland, Koźlice	51.105560°N, 14.983808°E	B. Tokarska-Guzik
1.3	<i>R. japonica</i>	Poland, Trzemienko	50.242639°N, 19.783250°E	B. Tokarska-Guzik
1.4	<i>R. japonica</i>	Poland, Poznań	52.409491°N, 16.984006°E	B. Wiatrowska
1.5	<i>R. japonica</i>	Poland, Pobiedziska	52.481637°N, 17.275364°E	B. Wiatrowska
1.6	<i>R. japonica</i>	Poland, Pobiedziska	52.480329°N, 17.284132°E	B. Wiatrowska
1.7	<i>R. japonica</i>	Poland, Gorzkie Pole	52.480755°N, 17.210946°E	B. Wiatrowska
1.8	<i>R. japonica</i>	Poland, Rakoniewice	52.136650°N, 16.271461°E	B. Wiatrowska
1.9	<i>R. japonica</i>	Poland, Lubięcín	51.886196°N, 15.872431°E	B. Wiatrowska
1.10	<i>R. japonica</i>	Poland, Głuszyca Górna	50.661948°N, 16.367022°E	P. Kiciński
1.11	<i>R. japonica</i>	Poland, Kowary	50.798055°N, 15.843444°E	Z. Dajdok
1.12	<i>R. japonica</i>	Poland, Wrocław Biskupin	51.096777°N, 17.110611°E	Z. Dajdok
1.13	<i>R. japonica</i>	Poland, Bolesławiec	51.267138°N, 15.554166°E	Z. Dajdok
1.14	<i>R. japonica</i>	Poland, Ząbkowice Śląskie	50.589527°N, 16.806916°E	Z. Dajdok
1.15	<i>R. japonica</i>	Poland, Oława	50.608083°N, 17.307500°E	Z. Dajdok
1.16	<i>R. japonica</i>	Poland, Wrocław-Żernik	51.124361°N, 16.915055°E	Z. Dajdok
1.17	<i>R. japonica</i>	Poland, Potasznia	51.539527°N, 17.493805°E	Z. Dajdok
1.18	<i>R. japonica</i>	Poland, Sławoszowice	51.533000°N, 17.307638°E	Z. Dajdok
1.19	<i>R. japonica</i>	Poland, Żmigródek	51.486388°N, 16.917222°E	Z. Dajdok
1.20	<i>R. japonica</i>	Poland, Trzebnica	51.319916°N, 17.059944°E	Z. Dajdok

Sample No.	Species	Localities	Geographical coordinates	Collector
1.21	<i>R. japonica</i>	Poland, Namysłów	51.080666°N, 17.710527°E	Z. Dajdok
1.22	<i>R. japonica</i>	Poland, Markowe	50.989750°N, 17.972666°E	Z. Dajdok
1.23	<i>R. japonica</i>	Poland, Brzeg	50.846361°N, 17.458361°E	Z. Dajdok
1.24	<i>R. japonica</i>	Poland, Wołczyn	51.010944°N, 18.068000°E	Z. Dajdok
1.25	<i>R. japonica</i>	Poland, Wiry	50.843611°N, 16.636972°E	Z. Dajdok
1.26	<i>R. japonica</i>	Poland, Wrocław-Strachocin	51.104166°N, 17.146944°E	Z. Dajdok
1.27	<i>R. japonica</i>	Poland, Solniki Wielkie	51.162808°N, 17.475097°E	Z. Dajdok
1.28	<i>R. japonica</i>	Poland, Ligota	49.885748°N, 18.947847°E	K. Bzdęga
1.29	<i>R. japonica</i>	Poland, Dzbanów	50.509698°N, 16.786601°E	K. Bzdęga
1.30	<i>R. japonica</i>	Poland, Dąbrowa Górnicza	50.316544°N, 19.229872°E	K. Bzdęga
1.31	<i>R. japonica</i>	Poland, Sucha Beskidzka	49.748925°N, 19.605571°E	K. Bzdęga
1.32	<i>R. japonica</i>	Poland, Zator	49.961216°N, 19.440823°E	K. Bzdęga
1.33	<i>R. japonica</i>	Poland, Szczekociny	50.637709°N, 19.755217°E	K. Bzdęga
1.34	<i>R. japonica</i>	Poland, Stare Czarnowo	53.324489°N, 14.684350°E	A. Gobber
1.35	<i>R. japonica</i>	Poland, Szczecin	53.366469°N, 14.729677°E	A. Gobber
1.36	<i>R. japonica</i>	Poland, Szczecin	53.389201°N, 14.514944°E	A. Gobber
1.37	<i>R. japonica</i>	Poland, Szczecin	53.408924°N, 14.558056°E	A. Gobber
1.38	<i>R. japonica</i>	Poland, Szczecin	53.361887°N, 14.737131°E	A. Gobber
1.39	<i>R. japonica</i>	Poland, Wrzosowo	54.013266°N, 14.804340°E	A. Gobber
1.40	<i>R. japonica</i>	Poland, Wolin	53.851410°N, 14.616641°E	A. Gobber
1.41	<i>R. japonica</i>	Poland, Skoszewo	53.781858°N, 14.631426°E	A. Gobber
2.1	<i>R. ×bohemica</i>	Poland, Poznań	52.407935°N, 16.956244°E	B. Wiatrowska
2.2	<i>R. ×bohemica</i>	Poland, Poznań	52.420542°N, 16.895445°E	B. Wiatrowska
2.3	<i>R. ×bohemica</i>	Poland, Poznań	52.433327°N, 16.911499°E	B. Wiatrowska
2.4	<i>R. ×bohemica</i>	Poland, Poznań	52.387607°N, 16.979838°E	B. Wiatrowska
2.5	<i>R. ×bohemica</i>	Poland, Poznań	52.418498°N, 16.907190°E	B. Wiatrowska
2.6	<i>R. ×bohemica</i>	Poland, Poznań	52.404153°N, 17.034627°E	B. Wiatrowska
2.7	<i>R. ×bohemica</i>	Poland, Poznań	52.420007°N, 16.959805°E	B. Wiatrowska
2.8	<i>R. ×bohemica</i>	Poland, Promno	52.475986°N, 17.209989°E	B. Wiatrowska
2.9	<i>R. ×bohemica</i>	Poland, Biskupice	52.460256°N, 17.179204°E	B. Wiatrowska
2.10	<i>R. ×bohemica</i>	Poland, Polanica Zdrój	50.417016°N, 16.511467°E	B. Tokarska-Guzik
2.11	<i>R. ×bohemica</i>	Poland, Skoczów	49.813271°N, 18.792147°E	B. Tokarska-Guzik
2.12	<i>R. ×bohemica</i>	Poland, Wilkanów	50.233900°N, 16.726300°E	B. Tokarska-Guzik
2.13	<i>R. ×bohemica</i>	Poland, Wilkowisko	49.754918°N, 20.259190°E	B. Tokarska-Guzik
2.14	<i>R. ×bohemica</i>	Poland, Radomierzyce	51.062083°N, 14.967150°E	B. Tokarska-Guzik
2.15	<i>R. ×bohemica</i>	Poland, Piskórka	51.988614°N, 21.020531°E	B. Tokarska-Guzik
2.16	<i>R. ×bohemica</i>	Poland, Izabelin	52.285350°N, 20.835167°E	B. Tokarska-Guzik
2.17	<i>R. ×bohemica</i>	Poland, Janowice Wielkie	50.880944°N, 15.925055°E	Z. Dajdok
2.18	<i>R. ×bohemica</i>	Poland, Bardo Śląskie	50.509444°N, 16.734000°E	Z. Dajdok
2.19	<i>R. ×bohemica</i>	Poland, Mościsko	50.782333°N, 16.588305°E	Z. Dajdok
2.20	<i>R. ×bohemica</i>	Poland, Wrocław-Biskupin	51.767222°N, 17.077777°E	Z. Dajdok
2.21	<i>R. ×bohemica</i>	Poland, Wrocław-Jarnołtów	51.123388°N, 16.841805°E	Z. Dajdok
2.22	<i>R. ×bohemica</i>	Poland, Międzyrzecze Górne	49.850545°N, 18.926569°E	K. Bzdęga
2.23	<i>R. ×bohemica</i>	Poland, Dąbrowa Górnicza	50.351958°N, 19.383935°E	K. Bzdęga
2.24	<i>R. ×bohemica</i>	Poland, Katowice	50.231923°N, 19.027429°E	K. Bzdęga
3.1	<i>R. sachalinensis</i>	Poland, Poznań	52.440737°N, 16.901670°E	B. Wiatrowska
3.2	<i>R. sachalinensis</i>	Poland, Poznań	52.439741°N, 16.910695°E	B. Wiatrowska
3.3	<i>R. sachalinensis</i>	Poland, Wierzonka	52.486721°N, 17.091999°E	B. Wiatrowska
3.4	<i>R. sachalinensis</i>	Poland, Istebna	49.603850°N, 18.902867°E	B. Tokarska-Guzik
3.5	<i>R. sachalinensis</i>	Poland, Smoleń	50.428804°N, 19.666947°E	B. Tokarska-Guzik
3.6	<i>R. sachalinensis</i>	Poland, Duszniki Zdrój	50.404733°N, 16.398750°E	B. Tokarska-Guzik

Sample No.	Species	Localities	Geographical coordinates	Collector
3.7	<i>R. sachalinensis</i>	Poland, Miedzianka	50.875833°N, 15.945100°E	B. Tokarska-Guzik
3.8	<i>R. sachalinensis</i>	Poland, Olszany	49.747900°N, 22.634933°E	B. Tokarska-Guzik
3.9	<i>R. sachalinensis</i>	Poland, Nowy Żytnik	54.252116°N, 16.686833°E	B. Tokarska-Guzik
3.10	<i>R. sachalinensis</i>	Poland, Boguszów-Gorce	50.751972°N, 16.182055°E	Z. Dajdok
3.11	<i>R. sachalinensis</i>	Poland, Karpacz	50.771305°N, 15.759416°E	Z. Dajdok
3.12	<i>R. sachalinensis</i>	Poland, Wrocław-Strachocin	51.103083°N, 17.143583°E	Z. Dajdok
3.13	<i>R. sachalinensis</i>	Poland, Łagiewniki	50.793527°N, 16.994555°E	Z. Dajdok
3.14	<i>R. sachalinensis</i>	Poland, Grodziszczce	50.797305°N, 16.565166°E	Z. Dajdok
3.15	<i>R. sachalinensis</i>	Poland, Wrocław-Żerniki	51.122333°N, 16.935555°E	Z. Dajdok
3.16	<i>R. sachalinensis</i>	Poland, Staroścín	50.986194°N, 17.838944°E	Z. Dajdok
3.17	<i>R. sachalinensis</i>	Poland, Dąbrowa	50.971361°N, 17.796166°E	Z. Dajdok
3.18	<i>R. sachalinensis</i>	Poland, Międzyrzecze Górne	49.850363°N, 18.926083°E	K. Bzdęga
3.19	<i>R. sachalinensis</i>	Poland, Sucha Beskidzka	49.748925°N, 19.605571°E	K. Bzdęga
3.20	<i>R. sachalinensis</i>	Poland, Katowice	50.231102°N, 19.026277°E	K. Bzdęga
3.21	<i>R. sachalinensis</i>	Poland, Stare Brynki	53.301346°N, 14.560283°E	A. Gobber
3.22	<i>R. sachalinensis</i>	Poland, Żabnica	53.295000°N, 14.501470°E	A. Gobber
3.23	<i>R. sachalinensis</i>	Poland, Miłachowo	53.939224°N, 14.775219°E	A. Gobber
3.24	<i>R. sachalinensis</i>	Poland, Police	53.539068°N, 14.577298°E	A. Gobber
3.25	<i>R. sachalinensis</i>	Poland, Szczecin	53.452032°N, 14.531967°E	A. Gobber
3.26	<i>R. sachalinensis</i>	Poland, Nowe Warpno	53.723634°N, 14.282981°E	A. Gobber
3.27	<i>R. sachalinensis</i>	Poland, Mierzeszyn	54.199942°N, 18.418546°E	A. Gobber
3.28	<i>R. sachalinensis</i>	Poland, Egierkowo	54.237831°N, 18.200722°E	A. Gobber
3.29	<i>R. sachalinensis</i>	Poland, Mały Klincz	54.131388°N, 18.049467°E	A. Gobber
3.30	<i>R. sachalinensis</i>	Poland, Piaszno	54.098524°N, 17.359810°E	A. Gobber

Appendix 2

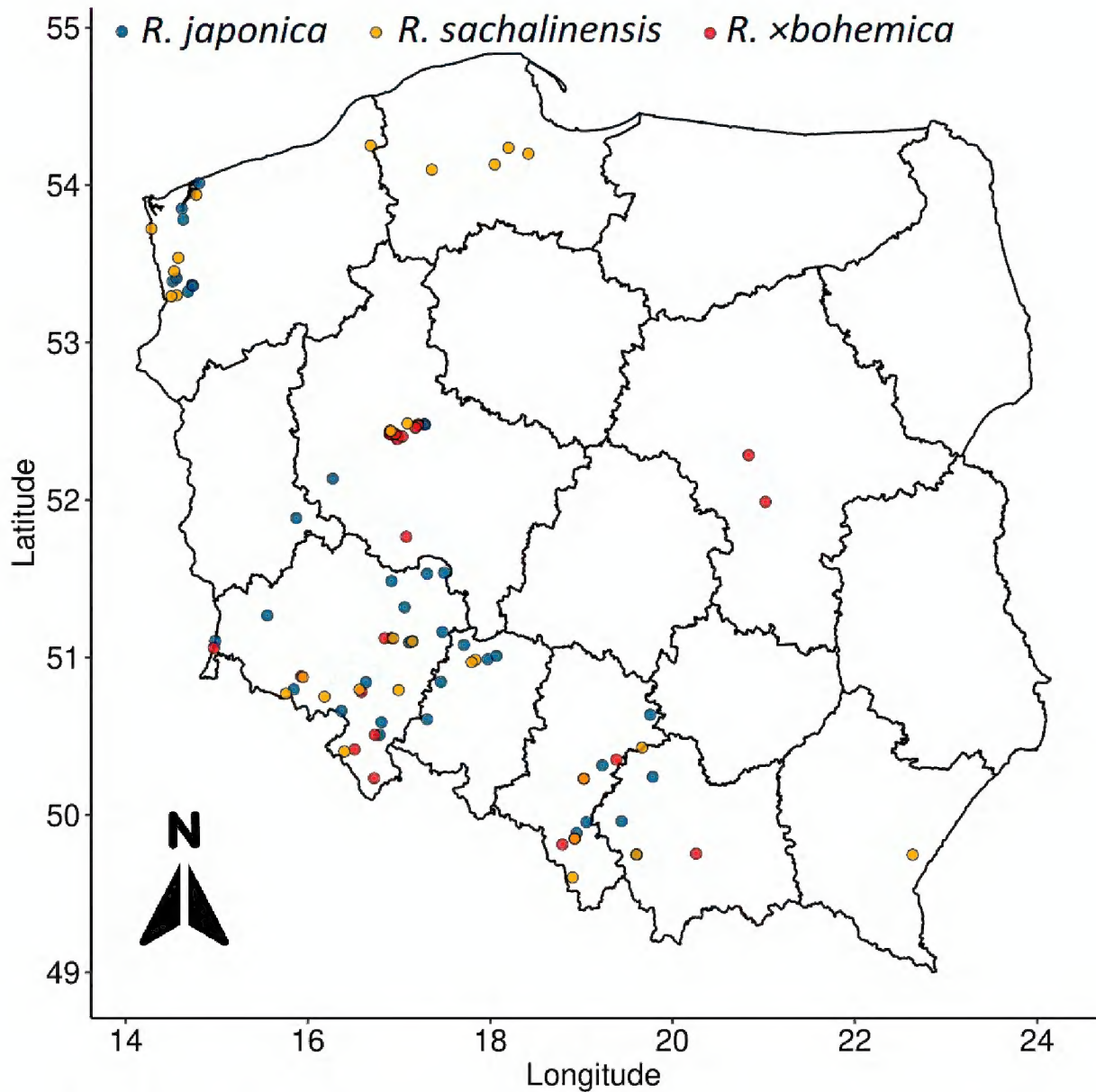


Figure A1. Localities of the *R. japonica*, *R. sachalinensis* and *R. xbohemica* sites in the study area.